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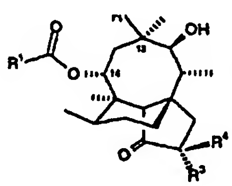
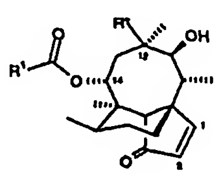
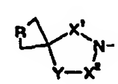


(IA)



(IB)



(II)

(57) Abstract

A compound of formula (IA) or (IB): in which R^1 is $R^A(CH_2)_nO(CH_2)_m$, $R^A(CH_2)_p$, Formula (II) or in which R is a spiro-fused mono- or bi-cyclic ring containing one or two basic nitrogen atoms; and X^1 and X^2, which may be the same or different, are each $-CH_2-$ or $-C{=}O$, provided that at least one of X^1 and X^2 is $-C{=}O$; Y is $-NH-$, $-CH_2-$ or $-CH_2-CH_2-$; R^A is an optionally substituted aryl group or heteroaryl group linked via a carbon atom; m is 1, 2 or 3; n is 0, 1 or 2; and p is 1 to 4; R^2 is vinyl or ethyl; R^3 is H, OH or F, and R^4 is H, or R^3 is H and R^4 is F. The compounds are useful for treating microbial infections in animals, especially in humans and in domesticated mammals.

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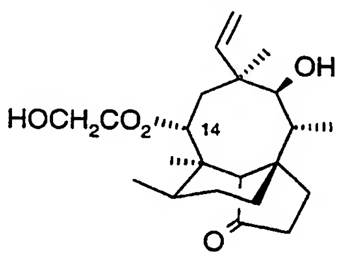
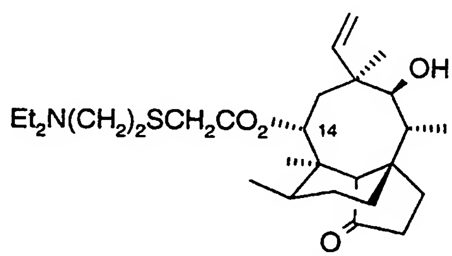
MUTILIN COMPOUNDS

The present invention relates to novel compounds, to processes for their preparation, to pharmaceutical compositions containing them and to their use in medical therapy, particularly antibacterial therapy.

Pleuromutilin, the compound of formula (A), is a naturally occurring antibiotic which has antimycoplasmal activity and modest antibacterial activity. It has been shown that the antimicrobial activity can be improved by replacing the glycolic ester moiety at position 14 by another acyloxy group R-X-CH_2CO_2-, where R is an aliphatic or aromatic moiety and X is O, S, or NR' (H. Egger and H. Reinshagen, *J. Antibiotics*, 1976, 29, 923). Tiamulin, the compound of formula (B), which is used as a veterinary antibiotic, is a derivative of this type (G. Hogenauer in *Antibiotics*, Vol. V, part 1, ed. F.E. Hahn, Springer-Verlag, 1979, p.344).



(A)



(B)

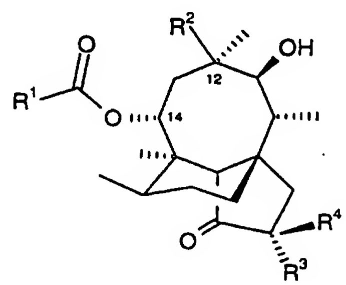
In this application, the non-conventional numbering system which is generally used in the literature (G. Hogenauer, *loc.cit.*) is used.

WO 97/25309 (SmithKline Beecham) describes further modification of the acyloxy group, disclosing 14-*O*-carbamoyl derivatives of mutilin or 19, 20-dihydromutilin, in which the N-atom of the carbamoyl group is unsubstituted, mono- or di-substituted.

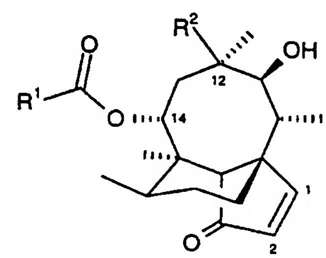
WO98/05659 (SmithKline Beecham) discloses 14-*O*-carbamoyl derivatives of mutilin or 19, 20-dihydromutilin, in which the N-atom of the carbamoyl group is acylated by a group which includes an azabicyclic moiety.

The present invention is based on the unexpected discovery that novel mutilin derivatives having either a spirocyclic acylcarbamate, an (hetero)arylalkyl carboxylate or an arylalkoxyalkyl carboxylate substituent at the 14-position also have antimicrobial activity.

Accordingly the present invention provides a compound of formula (IA) or (IB)



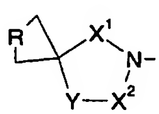
(IA)



(IB)

in which:

R^1 is $R^A(CH_2)_nO(CH_2)_m$, $R^A(CH_2)_p$, or



;

in which:

R is a spiro-fused mono- or bi-cyclic ring containing one or two basic nitrogen atoms; and

X^1 and X^2, which may be the same or different, are each $-CH_2-$ or -C=O, provided that at least one of X^1 and X^2 is -C=O;

Y is -NH-, $-CH_2-$ or $-CH_2-CH_2-$;

R^A is an optionally substituted aryl group or heteroaryl group linked via a carbon atom;

m is 1, 2 or 3;

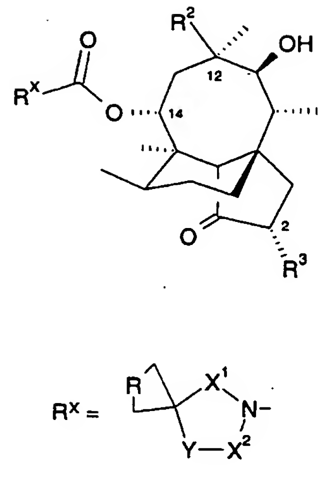
n is 0, 1 or 2; and

p is 1 to 4;

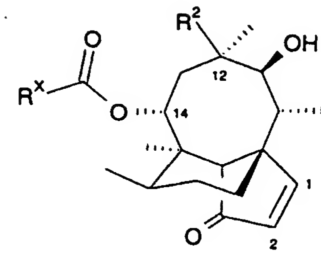
R^2 is vinyl or ethyl;

R^3 is H, OH or F, and R^4 is H, or R^3 is H and R^4 is F.

Within the compounds of formula (IA) and (IB) there exists a first class of compounds of formula (IA^1) or (IB^1):



(IA¹)



(IB¹)

in which:

R^2 is vinyl or ethyl;

R^3 is H, OH or F;

X^1 and X^2 which may be the same or different are each -CH_2- or -C=O, provided that at least one of X^1 and X^2 is -C=O;

Y is -NH-, -CH_2- or -CH_2-CH_2-; and

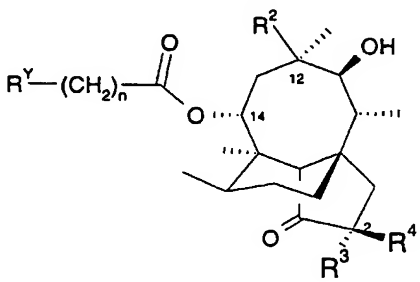
R is a spiro-fused monocyclic or bicyclic ring containing one or two basic nitrogen atoms.

When R is monocyclic it may contain from 4 to 8 ring atoms, and when bicyclic may contain from 5 to 10 ring atoms in each ring, and is optionally substituted on carbon by up to 3 substituents. Suitable substituents include alkyl, alkyloxy, alkenyl and alkenyloxy, each of which may be carried by either a bridgehead or a non-bridgehead carbon atom. In addition, the or each nitrogen atom in R may be substituted by oxygen to form an N-oxide, or by mono- or dialkyl, in which case it will be appreciated that a quaternary cation can be formed, or by alkoxycarbonylalkyl or by –C(=NR)NR'R" or –C(R)=NR' such that N is part of a guanidine or amidine functionality. The counterion may be a halide ion such as chloride or bromide, preferably chloride. The aza ring system additionally may contain one or more double bonds.

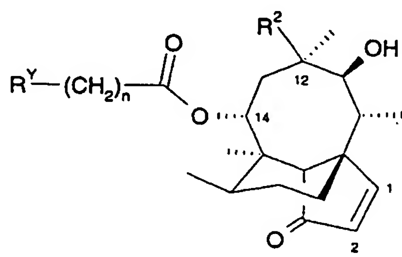
Examples of rings R that may be spiro-fused include piperidine and 1-azabicyclo-[2.2.1]-heptane, optionally N-substituted by (C_{1-6})alkyl, such as methyl, or (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, such as *t*-butyloxycarbonylmethyl, and quinuclidine.

The ring containing X^1, X^2 and Y is suitably pyrrolidinone, piperidinone or imidazolidone or the corresponding dione.

Within the compounds of formula (IA) and (IB) there also exists a second class of compounds of formula (IA^2) or (IB^2):



(IA^2)



(IB^2)

in which:

R^Y is an optionally substituted aryl group or heteroaryl group linked via a carbon atom;

R^2 is vinyl or;

R^3 is H, OH or F, and R^4 is H, or R^3 is H and R^4 is F; and

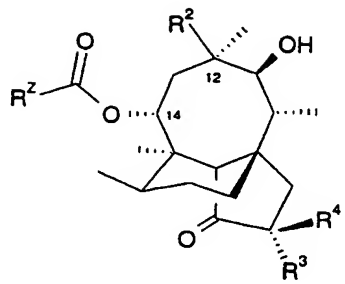
n = 1 to 4.

Preferably n is 1 or 2, and especially 1.

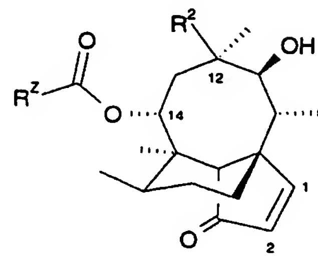
Representative values for R^Y when it is an aryl group include optionally substituted phenyl.

Representative values for R^Y when it is a heteroaryl group include optionally substituted thienyl, pyridinyl, furyl, thiazolyl, isoxazolyl, benzimidazolyl, quinolinyl, 1,2,3,4-tetrahydro-isoquinolinyl and benzothienyl.

Within the compounds of formula (IA) and (IB) there also exists a third class of compounds of formula (IA^3) or (IB^3):



(IA^3)



(IB^3)

in which:

R^Z is a group $R^Y(CH_2)_nO(CH_2)_m$;

in which:

R^Y is an optionally substituted aryl or heteroaryl group linked via a carbon atom;

n is 0, 1 or 2; and

m is 1, 2 or 3;

R^2 is vinyl or ethyl,

R^3 is H, OH or F, and R^4 is H or R^3 is H and R^4 is F.

In a preferred group of compounds R^Z is a group R^YOCH_2- , *i.e.* n is 0 and m is 1.

Representative values for R^Y when it is an aryl group include optionally substituted phenyl.

Representative values for R^Y when it is a heteroaryl group include optionally substituted thienyl, pyridinyl, furyl, thiazolyl, isoxazolyl, benzimidazolyl, quinolinyl, 1,2,3,4-tetrahydro-isoquinolinyl and benzothienyl.

In compounds of formula (IA^2), (IB^2), (IA^3) and (IB^3), the aryl or heteroaryl group is optionally substituted, typically by up to three substituents on carbon atoms. Representative substituents include nitro; optionally substituted (C_{1-6})alkyl, such as methyl optionally substituted by di(C_{1-6})alkylamino, cyano, carbamoyl, and N-containing non-aromatic heterocyclyl; and substituents containing primary, secondary and tertiary amines, including (C_{1-6})alkylamino(C_{1-6})alkyl and di(C_{1-6})alkylamino(C_{1-6})alkyl, such as dimethylaminomethyl, (C_{1-6})alkyl-and di(C_{1-6})alkyl-amino(C_{1-6})alkoxy, N-containing non-aromatic heterocyclyl, such as piperidinyl, (C_{1-6})alkylpiperidinyl(C_{1-6})alkyloxy, such as methylpiperidinylmethoxy, (C_{1-6})alkyl- and di(C_{1-6})alkyl-amino(C_{1-6})alkylcarbamyl, carbamimidoyl, and amidino. Other,

suitable substituents include alkyloxy, alkenyl and alkenyloxy. In addition, nitrogen atoms in heterocylic groups may be substituted by oxygen to form an N-oxide, or by mono- or dialkyl, in which case it will be appreciated that a quaternary cation can be formed. The counterion may be a halide ion such as chloride or bromide, preferably chloride. Preferred substituents include those containing primary, secondary and tertiary amines.

Alkyl and alkenyl groups referred to herein (individually or as part of alkoxy or alkenyloxy) may be straight and branched groups containing up to six carbon atoms and are optionally substituted by one or more groups selected from the group consisting of aryl(C_{1-6})alkoxy, aryl(C_{1-6})alkylthio, cycloalkyl, cycloalkenyl, carboxy and salts and esters thereof, halogen, hydroxy, (C_{1-6})alkoxy, aryloxy, (C_{1-6})alkoxycarbonyl, carbamoyl, mono- or di(C_{1-6})alkylcarbamoyl, sulphamoyl, mono- and di(C_{1-6})alkylsulphamoyl, amino, mono- and di(C_{1-6})alkylamino, (C_{1-6})acylamino, ureido, (C_{1-6})alkoxycarbonylamino, aryl, heterocyclyl, oxo, hydroxyimino, acyl, (C_{1-6})alkylthio, arylthio, (C_{1-6})alkane-sulphinyl, arylsulphinyl, (C_{1-6})alkanesulphonyl, arylsulphonyl, amidino, amidoxime and guanidino.

Cycloalkyl and cycloalkenyl groups referred to herein include groups having from three to eight ring carbon atoms and are optionally substituted as described hereinabove for alkyl and alkenyl groups.

When used herein, the term "aryl" means single and fused rings suitably containing from 4 to 7, preferably 5 or 6, ring atoms in each ring which rings may each be unsubstituted or substituted by, for example, up to three substituents. A fused ring system may include aliphatic rings and need include only one aromatic ring. Suitable aryl groups include phenyl and naphthyl such as 1-naphthyl or 2-naphthyl.

Suitably any aryl group, including phenyl and naphthyl, may be optionally substituted by up to five, preferably up to three substituents. Suitable substituents include halogen, (C_{1-6})alkyl, aryl, aryl(C_{1-6})alkyl, (C_{1-6})alkoxy, (C_{1-6})alkoxy(C_{1-6})alkyl, halo(C_{1-6})alkyl, aryl(C_{1-6})alkoxy, hydroxy, nitro, cyano, azido, amino, mono- and di-*N*-(C_{1-6})alkylamino, acylamino, arylcarbonylamino, acyloxy, carboxy, carboxy salts, carboxy esters, carbamoyl, mono- and di-*N*-(C_{1-6})alkylcarbamoyl, (C_{1-6})alkoxycarbonyl, aryloxycarbonyl, ureido, guanidino, sulphonylamino, aminosulphonyl, (C_{1-6})alkylthio, (C_{1-6})alkyl sulphinyl, (C_{1-6})alkylsulphonyl,

heterocyclyl and heterocyclyl (C_{1-6})alkyl. In addition, two adjacent ring carbon atoms may be linked by a (C_{3-5})alkylene chain, to form a carbocyclic ring.

When used herein the terms "heterocyclyl" and "heterocyclic" suitably include, unless otherwise defined, aromatic and non-aromatic, single and fused, rings suitably containing up to four heteroatoms in each ring, each of which is selected from oxygen, nitrogen and sulphur, which rings, may be unsubstituted or substituted by, for example, up to three substituents. Each heterocyclic ring suitably has from 4 to 7, preferably 5 or 6, ring atoms. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring.

When used herein, the term "heteroaryl" refers to a mono- or bicyclic heteroaromatic ring system comprising up to four, preferably 1 or 2, heteroatoms each selected from oxygen, nitrogen and sulphur. Each ring may have from 4 to 7, preferably 5 or 6, ring atoms. A bicyclic heteroaromatic ring system may include a carbocyclic ring.

Preferably a substituent for a heterocyclyl or a heteroaryl group is selected from halogen, (C_{1-6})alkyl, aryl(C_{1-6})alkyl, (C_{1-6})alkoxy, (C_{1-6})alkoxy(C_{1-6})alkyl, halo(C_{1-6})alkyl, hydroxy, amino, mono- and di-*N*-(C_{1-6})alkyl-amino, acylamino, carboxy, carboxy salts, carboxy esters, carbamoyl, mono- and di-*N*-(C_{1-6})alkylcarbonyl, aryloxycarbonyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, aryl, oxy groups, ureido, (C_{1-6})alkylguanidino, amidino, (C_{1-6})alkylamidino, sulphonylamino, aminosulphonyl, (C_{1-6})alkylthio, (C_{1-6})alkylsulphinyl, (C_{1-6})alkylsulphonyl, heterocyclyl and heterocyclyl(C_{1-6})alkyl.

Depending on the position of spiro attachments or of substituents, two or more diastereoisomers may be possible. The present invention includes the individual diastereoisomers and mixtures thereof.

It will be appreciated that the 2-substituted compounds of formula (IA^1) are of (2*S*) configuration.

The 2-hydroxy-substituted compounds of formula (IA^2) and (IA^3) are of the (2*S*) configuration. The 2-F-substituted compounds of formula (IA^2) and (IA^3) may be of (2*S*) configuration or (2*R*) configuration, or be provided as mixtures thereof. The (2*S*) configuration is however preferred.

Examples of compounds of the invention include:

2,8-diaza-8-methyl-1-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,9-diaza-9-methyl-1-oxospiro[5.5]undecane-2-carboxylic acid mutilin 14-ester;
2,4,8-triaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-8-methyl-3-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-1-oxospiro [4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-8-*tert*-butyloxycarbonylmethyl-1-oxospiro[4.5]decane-2carboxylic acid mutilin 14-ester;
(3*R*,4*S*)-spiro[(1-azabicyclo[2,2,1]heptane-3,3'-(2'-oxo-pyrrolidine)]-1'-carboxylic acid mutilin 14-ester;
(3*R*,4*S*)-spiro[(1-azabicyclo[2,2,1]-heptane)-3,3'-(2'-oxopyrrolidine)]-1'-carboxylic acid (2*S*)-2-hydroxy-mutilin 14-ester;
Mutilin 14-[4-(1-Methylpiperidin-4-ylmethyloxy)]phenylacetate;
Mutilin 14-(5-Dimethylaminomethyl-2-furyl)acetate;
Mutilin 14-[2-(piperidin-4-yl)thiazol-4-yl]acetate;
Mutilin 14-(4-dimethylaminomethylphenyl)acetate;
Mutilin 14-[3-(dimethylaminomethyl)phenoxy]acetate;
Mutilin 14-[4-(dimethylaminomethyl)phenoxy]acetate;
Mutilin 14-[4-(2-dimethylaminoethyl)phenoxy]acetate;
Mutilin 14-[4-(3-dimethylaminopropyl)phenoxy]acetate;
Mutilin 14-[4-(2-piperidin-1-yl-ethyl)phenoxy]acetate;
Mutilin 14-(2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yloxy)acetate; and
Mutilin 14-[4-(2-dimethylaminoethylaminocarbonyl)phenoxy]acetate.

The compounds of this invention may be in crystalline or non-crystalline form and, if crystalline, may optionally be hydrated or solvated. This invention includes within its scope stoichiometric hydrates as well as compounds containing variable amounts of water.

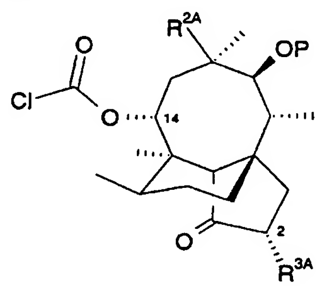
The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight.

The compounds of the invention may be in the form of free bases or acid addition salts. Examples carrying a carboxy substituent may be in the form of zwitterions, or alkali metal salts (of the carboxy group). Pharmaceutically acceptable salts are preferred.

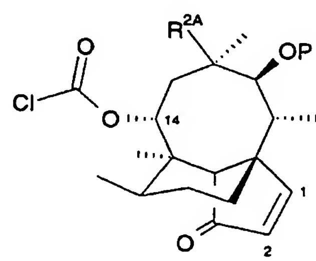
Pharmaceutically acceptable acid-addition salts include those described by Berge, Bighley, and Monkhouse, *J. Pharm. Sci.*, 1977, **66**, 1-19. Suitable salts include the hydrochloride, maleate, and methanesulphonate; particularly the hydrochloride.

The present invention also provides methods for preparing the compounds of the invention, starting from mutilin or *epi*-mutilin and introducing the C-14 side chain by an appropriate coupling reaction. The mutilin nucleus may be modified to introduce 2-F; 2-OH; 19, 20-dihydro; or 1, 2-dehydro substituents, before or after the coupling.

More particularly, the present invention provides a method for preparing compounds of formula (IA^1) and (IB^1) which comprises reacting a compound of formula (IIA^1) or (IIB^1):

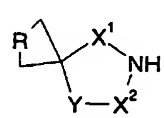


(IIA^1)



(IIB^1)

in which P is hydrogen or a removable hydroxy-protecting group, and R^{2A} and R^{3A} are R^2 and R^3 as defined for formulae (IA) and (IB) or groups convertible to R^2 and R^3, with a lactam of formula (III):

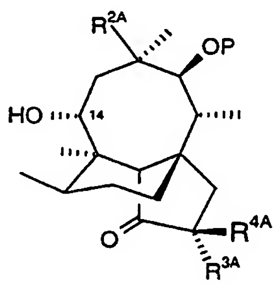


(III)

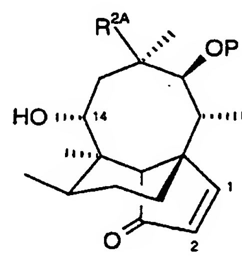
in which R, X^1, X^2 and Y are as defined for compounds of formula (IA^1) and (IB^1)

in the presence of a base (*e.g.* lithium di-*iso*-propylamide, lithium hexamethyl-disilazide), suitably in a non-protic solvent (*e.g.* tetrahydrofuran, 1,2-dimethoxyethane), typically at a temperature of -60°C to 0°C; and, where required or desired,
converting P to hydrogen,
converting an R^{2A} or R^{3A} group to a R^2 or R^3 group, and/or
converting one R^2 or R^3 group to another R^2 or R^3 group.

The present invention also provides a process for preparing a compound of formula (IA^2) or (IB^2) which comprises reacting a compound of formula (IIA^2) or (IIB^2):



(IIA^2)



(IIB^2)

where:
P is hydrogen or a removable hydroxy-protecting group, and R^{2A}, R^{3A} and R^{4A} are R^2, R^3 and R^4 as defined for formulae (IA) and (IB) or groups convertible to R^2, R^3 and R^4;
with an active derivative of a carboxylic acid of formula (IV):

$$R^{YA}\text{-}(CH_2)_n\text{-}COOH \quad \text{(IV)}$$

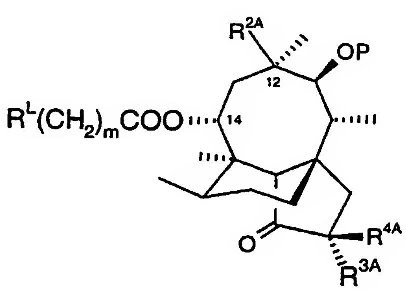
where;
R^{YA} is R^Y as defined for formulae (IA^2) and (IB^2) or a group convertible to R^Y;
under ester forming conditions and thereafter, if so needed;
converting P to hydrogen,
converting an R^{YA}, R^{2A}, R^{3A} or R^{4A} group to a R^Y, R^2, R^3 or R^4 group, and/or
converting one R^Y, R^2, R^3 or R^4 group to another R^Y, R^2, R^3 or R^4 group.

Compounds of formula (IA^2) and (IB^2) may also be prepared by coupling a mutilin having a carboxylate-forming group at position 14, such as the 14-chloroformate, with an appropriate organolithium compound, and if necessary converting the *epi*-mutilin to mutilin. Accordingly, in a further aspect, the present invention provides a process for preparing a compound of formula (IA^2) or (IB^2) which comprises reacting a compound of the formula (IIA^1) or (IIB^1) as hereinbefore defined;
with an organo-lithium compound of formula (VI):

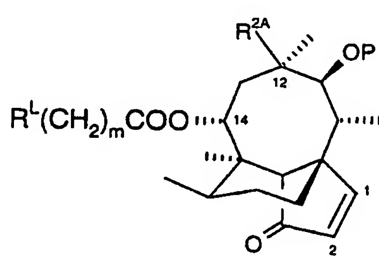
$$R^{YA}\text{-}(CH_2)_n\text{-}Li \qquad \text{(VI)}$$

suitably in a non-protic solvent, such as THF, typically at a temperature in the range –78 to 0°C;
and where required or desired;
converting P to hydrogen,
converting an R^{YA}, R^{2A}, R^{3A} or R^{4A} group to a R^Y, R^2, R^3 or R^4 group, and/or converting one R^Y, R^2, R^3 or R^4 group to another R^Y, R^2, R^3 or R^4 group.

The present invention additionally provides a process for preparing a compound of formula (IA^3) or (IB^3) which comprises reacting a compound of formula (IIA^3) or (IIB^3):



(IIA^3)



(IIB^3)

where:
P is hydrogen or a removable hydroxy-protecting group, and R^{2A}, R^{3A} and R^{4A} are R^2, R^3 and R^4 as defined for formulae (IA) and (IB) or groups convertible to R^2, R^3 and R^4, and R^L is a leaving group or OH ,
with:

(a) when R^L is a leaving group, a compound of the formula (VA):

$$R^{YA}\text{-}(CH_2)_n\text{-}Q \qquad \text{(VA)}$$

where R^{YA} is R^Y as defined for formulae (IA^3) and (IB^3) or a group convertible to R^Y, and Q is OH or an alkoxide derivative thereof;

(b) when R^L is hydroxy, a compound of the formula (VB):

$$R^{YA}\text{-}(CH_2)_n\text{-}Q^2 \qquad \text{(VB)}$$

where R^{YA} is R^Y as defined for formulae (IA^3) and (IB^3) or a group convertible to R^Y, and Q^2 is a leaving group;

(c) when R^L is hydroxy and n is 0, an alcohol R^{YA} OH where R^{YA} is R^Y as defined for formulae (IA^3) and (IB^3) or a group convertible to R^Y;

by one of the procedures set out below,

and thereafter, where required or desired,

converting P to hydrogen,

converting an R^{YA}, R^{2A}, R^{3A} or R^{4A} group to an R^Y, R^2, R^3 or R^4 group, and/or

converting one R^Y, R^2, R^3 or R^4 group to another R^Y, R^2, R^3 or R^4 group.

Suitable leaving groups for R^L and Q^2 include halogen, tosylate, mesylate, diazonium, etc.

Procedures for coupling the group $R^L(CH_2)_mCO.O$- with a compound of formula (VA/B) include the following:

(a) when R^L is a leaving group, such as tosyl, mesyl, chloro or bromo, by reaction of R^L with an alkoxide anion $R^{YA}(CH_2)_nO^-$ in a non-protic solvent (e.g. tetrahydrofuran or *N,N* dimethylformamide), typically by analogy to the method of H. Egger and H. Reinshagen, *J. Antibiot.*, 1976, **29**, 915, for example forming the anion *in situ* by reacting the corresponding alcohol with sodium hydride;

(b) when R^L is OH, reacting R^L with an electrophile containing the residue $R^{YA}(CH_2)_n$-, such as an aralkyl halide or aryl- or aralkyl-diazonium using conventional conditions for electrophilic substitution;

(c) when R^L is OH and n is 0, reacting R^L with an alcohol $R^{YA}OH$ in a Mitsunobu reaction.

Compounds of the formula (IA^3) or (IB^3) may also be prepared by coupling a mutilin (having a protected hydroxy group at position 11), with an active derivative of a carboxylic acid $R(CH_2)_nO(CH_2)_mCOOH$, such as an acid chloride.

Accordingly, in a further aspect, the present invention provides a process for preparing a compound of formula (IA^3) or (IB^3) which comprises reacting a compound of formula (IIA^2) or (IIB^2) as hereinbefore defined;
with an active derivative of a carboxylic acid of formula (VII):

$$R^{YA}(CH_2)_nO(CH_2)_mCOOH \qquad \text{(VII)}$$

where n and m are as defined for compounds of formulae (IA^3) and (IB^3) and R^{YA} is R^Y as defined for formulae (IA^3) and (IB^3) or a group convertible to R^Y;
under ester forming conditions; and thereafter, where required or desired;
converting P to hydrogen,
converting an R^{YA}, R^{2A}, R^{3A} or R^{4A} group to an R^Y, R^2, R^3 or R^4 group, and/or converting one R^Y, R^2, R^3 or R^4 group to another R^Y, R^2, R^3 or R^4 group.

Conventional ester forming conditions are described in the literature, see for example as in *Comprehensive Organic Functional Group Transformations*, Vol. 5, ed. C.J. Moody, p. 123-130, Elsevier Scientific, Oxford, 1995. The active derivative used as an acylating agent may be an acid chloride, acid bromide, a mixed anhydride, or an *N*-acyl-imidazole. The preferred agent is an acid chloride. General methods for forming such acylating agents from the acid are described in the chemical literature (see I.O. Sutherland, *Comprehensive Organic Chemistry*, Vol. 2, ed. I.O. Sutherland, pages 875-883 (Pergamon Press, Oxford, 1979), and references therein).

Useful methods for acylating the 14-hydroxyl in the present invention include the use of the following:

acid chloride in *N,N*-dimethylformamide at elevated temperature (*e.g.* 100°C to 120°C);

acid chloride in the presence of an organic base (*e.g.* pyridine, 2,6-lutidine, 2,4,6-collidine, di-*iso*-propylethylamine) or an inorganic base (*e.g.* sodium or lithium hexamethyldisilazide);

carboxylic acid in the presence of dicyclohexylcarbodiimide and an acylation catalyst (*e.g.* 4-dimethylamino-pyridine, 4-pyrrolidino-pyridine); and

a mutilin 14-chloroformate derivative plus carboxylic acid, tertiary base (*e.g.* triethylamine, di-*iso*-propyl-ethylamine), and an acylation catalyst (*e.g.* 4-dimethylamino-pyridine, 4-pyrrolidino-pyridine).

It may also be necessary to protect substituent groups in compounds (III), (IV), (VA/B), (VI) and (VII) prior to the coupling reaction, for example protecting N atoms with alkoxycarbonyl, for example *t*-butoxycarbonyl.

Preferably P is a hydroxyl protecting group such as an acyl group, for example so that -OP is trifluoroacetyl or dichloroacetyl. When the intended R^3 is also hydroxyl then R^{3A} is also preferably acyloxy, for example acetyl or dichloroacetyl. Hydroxyl groups at positions 11 and 2 (as groups OP and R^{3A}) may be protected using, for example, dichloroacetic anhydride and pyridine in tetrahydrofuran or *N*-trifluoroacetyl-imidazole in tetrahydrofuran at 0°C. After the coupling reaction is complete the protecting acyl groups may be removed to restore the hydroxyl groups by hydrolysis *e.g.* using NaOH in MeOH.

Suitable hydroxy, carboxy and amino protecting groups are those well known in the art and which may be removed under conventional conditions and without disrupting the remainder of the molecule. A comprehensive discussion of the ways in which hydroxy, carboxy and amino groups may be protected and methods for cleaving the resulting protected derivatives is given in for example "Protective Groups in Organic Chemistry" (T.W. Greene, Wiley-Interscience, New York, 2nd edition, 1991). Particularly suitable hydroxy protecting groups include, for example, triorganosilyl groups such as, for instance, trialkylsilyl and also organocarbonyl and organooxycarbonyl groups such as, for instance, acetyl, allyloxycarbonyl, 4-methoxybenzyloxycarbonyl and 4-nitrobenzyloxycarbonyl. Particularly suitable carboxy protecting groups include alkyl and aryl groups, for instance methyl, ethyl and phenyl. Particularly suitable amino

protecting groups include alkoxycarbonyl, 4-methoxybenzyloxycarbonyl and 4-nitrobenzyloxycarbonyl.

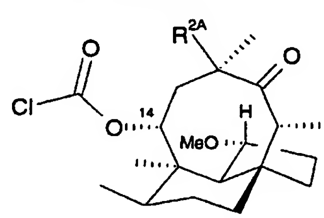
Conversions of an R^{YA}, R^{1A}, R^{2A}, R^{3A} or R^{4A} group to a R^Y, R^1, R^2, R^3 or R^4 group typically arise when a protecting group is needed during the above coupling reaction or during the preparation of the reactants by the procedures described below. Interconversion of one R^Y, R^1, R^2, R^3 or R^4 group to another typically arises when one compound of formula (I) is used as the immediate precursor of another compound of formula (I) or when it is easier to introduce a more complex or reactive substituent at the end of a synthetic sequence.

R^{2A} is typically the R^2 group vinyl, and this may be converted to the alternative R^2 ethyl group by hydrogenating the vinyl group to form an ethyl group, typically by hydrogenation over a palladium catalyst (*e.g.* 10% palladium-on-carbon) in a solvent such as ethyl acetate, ethanol, dioxane, or tetrahydrofuran.

R^{3A} is typically hydrogen, fluoro or protected hydroxyl, such as acyloxy. After the coupling reaction, protecting acyl groups may be removed to restore the hydroxyl groups by hydrolysis *e.g.* using NaOH in MeOH.

Compounds of formula (IA) may also be prepared from an *epi*-mutilin starting material.

This invention also provides for the preparation of a compound of formula (IA^1) by the reaction of a compound of formula (IIC^1):



(IIC^1)

where P and R^{2A} are as defined for formulae (IIA^1);

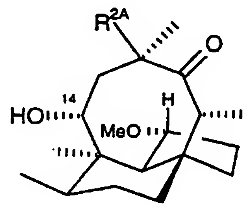
with the lactam of formula (III) as defined above

under similar conditions to the coupling of the compounds of formulae (IIA^1) and (IIB^1) with lactam (III), and then

treating the product with an acid,

and where required or desired
converting an R^{2A} group to a R^2 group, and/or
converting one R^2 group to another R^2 group.

A compound of formula (IA^2) may be prepared by reacting an epi-mutilin compound of formula (IIC^2):



(IIC^2)

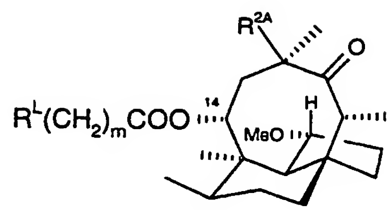
where:

R^{2A} is as defined for formulae (IIA^2) and (IIB^2);

with an active derivative of the acid of formula (IV) under ester forming conditions, as hereinbefore described, and then
treating the product with an acid,
and where required or desired
converting an R^{1A} or R^{2A} group to a R^1 or R^2 group, and/or
converting one R^1 or R^2 group to another R^1 or R^2 group.

Alternatively a compound of formula (IA^2) may be prepared by reacting an *epi*-mutilin compound of formula (IIC^1) as defined above;
with the organo-lithium compound of formula (VI) above, and then
treating the product with an acid,
and where required or desired
converting an R^{YA} or R^{2A} group to a R^Y or R^2 group, and/or
converting one R^Y or R^2 group to another R^Y or R^2 group.

A compound of formula (IA^3) may also be prepared from an *epi*-mutilin starting material, for instance by reacting a compound of formula (IIC^3):



(IIC^3)

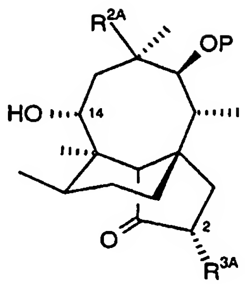
where R^{2A} and R^L are as defined for formulae (IIA^3) and (IIB^3); with the active derivative (V) by the procedures (a), (b) or (c) set out above, and then; treating the product with an acid, and thereafter and where required or desired; converting an R^{YA} or R^{2A} group to a R^Y or R^2 group, and/or converting one R^Y or R^2 group to another R^Y or R^2 group.

Additionally a compound of formula (IA^3) may also be prepared by reacting a compound of formula (IIC^2) as defined above; with an active derivative of a carboxylate acid of formula (VII) under ester forming conditions, as described above; and then, treating the product with an acid, and thereafter and where required or desired; converting an R^{YA} or R^{2A} group to an R^Y or R^2 group, and/or converting one R^Y or R^2 group to another R^Y or R^2 group.

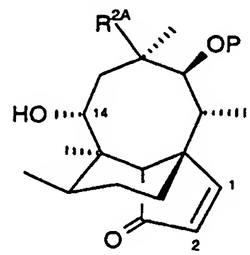
It will be appreciated that the acid treatment indicated above converts the *epi*-mutilin configuration of formula (IIC) to the usual mutilin nucleus of formula (IIA). Typically this conversion is carried out by treatment with conc. HCl or Lukas reagent (conc. HCl saturated with $ZnCl_2$) in dioxane.

R^{2A} is typically the R^2 group vinyl, and this may be converted to the alternative R^2 group by hydrogenating the vinyl group to form an ethyl group. Also it may again be necessary to protect substituent groups in compounds of formula (III), (IV), (VA/B), (VI) and (VII) prior to the coupling reaction, for example protecting N atoms with alkoxycarbonyl, for example *t*-butoxycarbonyl.

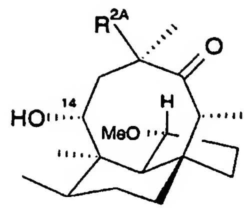
The compounds of formulae (IIA^1), (IIB^1) and (IIC^1) may be prepared by reacting the corresponding compounds of formula (IIA^2), (IIB^2) and (IIC^2) in which the substituent at position 11 is hydroxyl:



(IIA^2)



(IIB^2)



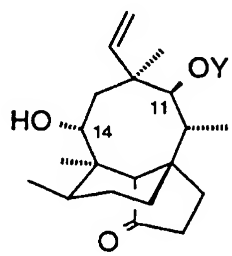
(IIC^2)

where P, R^{2A} and R^{3A} are as defined for formulae (IIA^1), (IIB^1) and (IIC^1); with phosgene or a phosgene equivalent, such as trichloromethyl chloroformate or bis(trichloromethyl) carbonate, in the presence of an organic base in a suitable solvent, such as tetrahydrofuran. Suitable bases include pyridine, 2,6-lutidine, triethylamine, and *N,N*-di-*iso*-propylethylamine. The reaction typically is carried out at 0°C to 20°C. For this reaction OP and R^{3A} hydroxyl groups are suitably protected as described above.

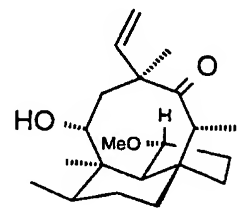
The compounds of formulae (IIA^3), (IIB^3) and (IIC^3) may be prepared from the corresponding compounds of formula (IIA^2), (IIB^2) and (IIC^2), using conventional methodology for introducing acyl groups substituted by hydroxyl or a leaving group, see for instance K. Riedl in *J. Antibiotics*, 1976, **29**, 132 (for the preparation of the chloride and tosylate, where m = 1); and H. Egger and H. Reinshagen in *J. Antibiotics*, 1976, **29**, 915 (tosylate and mesylate, for m = 1 starting from pleuromutilin or 19,20-dihydro-pleuromutilin). In addition, compounds where R^L is chloro or bromo may be prepared by reacting $Br(CH_2)_mCOOCl/Br$ or $Cl(CH_2)_mCOOCl/Br$ with compounds (VIII) and (IX) below. Compounds of formula (IIA^3) in which R^{3A} is hydroxyl or fluoro may be readily prepared from corresponding compounds of formula (IIA^3) in which R^{3A} is hydrogen,

via an intermediate 2-diazo compound, using procedures based on those described by G. Schulz and H. Berner in *Tetrahedron*, 1984, **40**, 905, for pleuromutilin.

Compounds of formulae (IIA^2), (IIB^2) and (IIC^2) may be prepared from compounds of formulae (VIII) and (IX):



(VIII)



(IX)

Suitable compounds of formula (VIII) include 11-O-acyl mutilin derivatives, e.g. mutilin 11-acetate (A.J. Birch, C.W. Holzapfel, R.W. Richards, *Tetrahedron* (Suppl.), 1966, 8, Part II, 359) or mutilin 11-dichloroacetate or mutilin 11-trifluoroacetate. Formula (IX) is (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-epi-mutilin (H. Berner, G. Schulz and H. Schneider, *Tetrahedron*, 1980, 36, 1807).

Compounds (VIII) and (IX) are effectively the compounds of formula (IIA^2) and (IIC^2) respectively in which R^{2A} is vinyl. They may be converted into the corresponding compounds in which R^{2A} is ethyl by hydrogenation, typically by hydrogenation over a palladium catalyst (*e.g.* 10% palladium-on-carbon) in a solvent such as ethyl acetate, ethanol, dioxane, or tetrahydrofuran.

Compounds of formula (IIA^2) in which R^{3A} is hydroxyl or fluoro may be prepared by first preparing 2-hydroxymethylene mutilin from a compound of formula (VIII). Using procedures based on that described by A.J. Birch, C.W. Holzapfel and R.W. Rickards, *Tetrahedron* (Suppl) 1996, 8, part III, 359), a compound of formula (VIII) in toluene and methyl formate is treated with sodium methoxide and stirred under argon. The product is a mixture of the desired 2-hydroxymethylene compound and corresponding compounds substituted by formate at position 11 (if OP is OH) and /or position 14. The formate groups may be removed when desired by treatment with potassium hydroxide in methanol.

However the product mixture may be used directly to prepare 2-diazo-mutilin derivatives using the method described by H. Berner, G. Schulz, and G. Fisher, *Monatsh. Chem.*, 1981, 112, 1441, for example by reacting a solution of a 2-hydroxymethylene-mutilin and the formate derivatives in dichloromethane at -10 °C under argon with tosyl azide and triethylamine. Removal of the formate groups as described above leaves 2-diazo-mutilin, which may be reacted with a carboxylic acid to give a 2-acyloxy-mutilin, effectively a compound of formula (IIA^2) in which R^{3A} is protected hydroxyl. Suitably reaction with dichloroacetic acid gives 2-dichloroacetoxy-mutilin, which can be deprotected as described above to provide the desired (2*S*)-2-hydroxy derivative, preferably after the coupling reaction.

Compounds of formula (IIA^2) in which R^{3A} is fluoro may be obtained by reacting 2-diazo-mutilin with a source of hydrogen fluoride. Conveniently, the hydrogen fluoride source is an amine complex of hydrogen fluoride such as hydrogen fluoride-pyridine. The reaction may be carried out in an anhydrous solvent (*e.g.* diethyl ether, tetrahydrofuran, 1,2-dimethoxyethane) at a temperature of -15°C to 25°C.

This reaction produces (2*S*)-2-fluoro derivatives. (2*R*)-2-Fluoro-mutilin derivatives may be prepared by treating the (2*S*)-isomer with a base (*e.g.* sodium hydroxide or potassium hydroxide in ethanol). This will usually produce a mixture of (2*S*) and (2*R*)-isomers that may be separated using conventional techniques such as chromatography and crystallisation.

Compounds of formula (IIB^2) are either 1,2-didehydro-mutilin or obtainable therefrom by manipulation of OP and R^{2A} as described above. 1,2-Didehydro-mutilins can be prepared using the method described by G. Schulz and H. Berner in *Tetrahedron*, 1984, 40, 905, by treating the 2-diazo compound with conc. HCl, to give the 2-chloro derivative and then eliminating HCl, by heating at 160°C in 2,6-lutidine.

Compounds of formulae (III), (IV), (VA/B) (VI) and (VII), are either commercially available or may be obtained therefrom using conventional synthetic methodology.

Where intermediates disclosed for the above processes are novel compounds, they also form part of this invention.

The compounds of the present invention may contain a chiral centre, and therefore the above processes may produce a mixture of diastereoisomers. A single

diastereoisomer may be prepared by separating such a mixture of diastereoisomers which has been synthesised using a racemic starting material, or by synthesis using an optically pure starting material.

The compounds of this invention may be in crystalline or non-crystalline form, and, if crystalline, may optionally be hydrated or solvated. When some of the compounds of this invention are allowed to crystallise or are recrystallised from organic solvents, solvent of crystallisation may be present in the crystalline product. Similarly, some of the compounds of this invention may be crystallised or recrystallised from solvents containing water. In such cases water of hydration may be present in the crystalline product. Crystallisation procedures will usually produce stoichiometric hydrates. Compounds containing variable amounts of water may be produced by processes such as lyophilisation.

The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight. An impure or less pure form of a compound according to the invention may, for example, be used in the preparation of a more pure form of the same compound or of a related compound (for example a corresponding derivative) suitable for pharmaceutical use.

The present invention also includes pharmaceutically acceptable salts and derivatives of the compounds of the invention. Salt formation may be possible when one of the substituents carries an acidic or basic group. Salts may be prepared by salt exchange in conventional manner.

Acid-addition salts may be pharmaceutically acceptable or non-pharmaceutically acceptable. In the latter case, such salts may be useful for isolation and purification of the compound of the invention, or intermediates thereto, and will subsequently be converted into a pharmaceutically acceptable salt or the free base.

The compounds of the present invention and their pharmaceutically acceptable salts or derivatives have antimicrobial properties and are therefore of use in therapy, in partiuclar for treating microbial infections in animals, especially mammals, including humans, in particular humans and domesticated animals (including farm animals). The compounds may be used for the treatment of infections caused by, for example, Gram-

positive and Gram-negative bacteria and mycoplasmas, including, for example, *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus agalactiae, Streptococcus pneumoniae, Haemophilius sp., Neisseria sp., Legionella sp., Chlamydia sp., Moraxella catarrhalis, Mycoplasma pneumoniae*, and *Mycoplasma gallisepticum*.

The present invention also provides a method of treating microbial infections in animals, especially in humans and in domesticated mammals, which comprises administering a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof, or a composition according to the invention, to a patient in need thereof.

The invention further provides the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the preparation of a medicament for use in the treatment of microbial infections.

Compounds of the present invention may be used to treat skin and soft tissue infections and acne, by topical application. Accordingly, in a further aspect the present invention provides the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the preparation of a medicament adapted for topical administration for use in the treatment of skin and soft tissue infections and also in the treatment of acne in humans.

Compounds of the present invention may be also used for the elimination or reduction of nasal carriage of pathogenic bacteria such as *S. aureus, H. influenzae, S. pneumonia* and *M. catarrhalis*, in particular colonisation of the nasospharynx by such organisms, by the administration of a compound of the present invention thereto. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a medicament adapted for administration to the nasal cavity, for reducing or eliminating the nasal carriage of pathogenic organisms. Preferably, the medicament is adapted for focussed delivery to the nasopharynx, in particular the anterior nasopharynx.

Such reduction or elimination of nasal carriage is believed to be useful in prophylaxis of recurrent acute bacterial sinusitis or recurrent otitis media in humans, in particular in reducing the number of episodes experienced by a patient over a given period of time or increasing the time intervals between episodes. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture

of a medicament adapted for administration to the nasal cavity, for prophylaxis of recurrent acute bacterial sinusitis or recurrent otitis media.

Compounds of the present invention are also useful in treating chronic sinusitis. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a medicament, for treating of chronic sinusitis.

The compounds according to the invention may suitably be administered to the patient at a daily dosage of from 1.0 to 50 mg/kg of body weight. For an adult human (of approximately 70 kg body weight), from 50 to 3000 mg, for example about 1500 mg, of a compound according to the invention may be administered daily. Suitably, the dosage for adult humans is from 5 to 20 mg/kg per day. Higher or lower dosages may, however, be used in accordance with normal clinical practice.

To lessen the risk of encouraging the development of resistant organisms during prophylaxis of recurrent otitis media or recurrent acute bacterial sinusitis, it is preferred to administer the drug on an intermittent, rather than a continual, basis. In a suitable intermittent treatment regimen for prophylaxis of recurrent otitis media or recurrent sinusitis, drug substance is administered on a daily basis, for a small number of days, for instance from 2 to 10, suitably 3 to 8, more suitably about 5 days, the administration then being repeated after an interval, for instance, on a monthly basis over a period of months, for instance up to six months. Less preferably, the drug substance may be administered on a continuing, daily basis, over a prolonged period, for instance several months. Suitably, for prophylaxis of recurrent otitis media or recurrent sinusitis, drug substance is administered once or twice a day. Suitably, drug substance is administered during the winter months when bacterial infections such as recurrent otitis media and recurrent sinusitis tend to be more prevalent. The drug substance may be administered at a dosage of from 0.05 to 1.00mg, typically about 0.1 to 0.2mg, in each nostril, once or twice a day.

More generally, the compounds and compositions according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antibiotics.

Accordingly, in a further aspect, the present invention provides a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof together with a pharmaceutically acceptable carrier or excipient.

The compounds and compositions according to the invention may be formulated for administration by any route, for example oral, topical or parenteral. The compositions may, for example, be made up in the form of tablets, capsules, powders, granules, lozenges, creams, syrups, sprays or liquid preparations, for example solutions or

suspensions, which may be formulated for oral use or in sterile form for parenteral administration by injection or infusion.

Tablets and capsules for oral administration may be in unit dosage form, and may contain conventional excipients including, for example, binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; and pharmaceutically acceptable wetting agents, for example sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations may contain conventional additives, including, for example, suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters (for example glycerine), propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and, if desired, conventional flavouring and colour agents.

Compositions according to the invention intended for topical administration may, for example, be in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, nose drops, nasal sprays, impregnated dressings, and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, ethanol or oleyl alcohol for lotions and aqueous bases for sprays. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

Compositions according to the invention intended for topical administration, in addition to the above, may also contain a steroidal anti-inflammatory agent; for example, betamethasone.

Compositions according to the invention may be formulated as suppositories, which may contain conventional suppository bases, for example cocoa-butter or other glycerides.

Compositions according to the invention intended for parenteral administration may conveniently be in fluid unit dosage forms, which may be prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, may be either suspended or dissolved in the vehicle. In preparing solutions, the compound may be dissolved in water for injection and filter-sterilised before being filled into a suitable vial or ampoule, which is then sealed. Advantageously, conventional additives including, for example, local anaesthetics, preservatives, and buffering agents can be dissolved in the vehicle. In order to enhance the stability of the solution, the composition may be frozen after being filled into the vial, and the water removed under vacuum; the resulting dry lyophilised powder may then be sealed in the vial and a accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions may be prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may instead be sterilised by exposure to ethylene oxide before being suspended in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in such suspensions in order to facilitate uniform distribution of the compound.

A compound or composition according to the invention is suitably administered to the patient in an antimicrobially effective amount.

A composition according to the invention may suitably contain from 0.001% by weight, preferably (for other than spray compositions) from 10 to 60% by weight, of a compound according to the invention (based on the total weight of the composition), depending on the method of administration.

When the compositions according to the invention are presented in unit dosage form, for instance as a tablet, each unit dose may suitably comprise from 25 to 1000 mg, preferable from 50 to 500 mg, of a compound according to the invention.

Representative compositions of the present invention include those adapted for intranasal administration, in particular, those that will reach into the nasopharynx. Such compositions are preferably adapted for focussed delivery to, and residence within, the nasopharynx. The term 'focussed delivery' is used to mean that the composition is delivered to the nasopharynx, rather than remaining within the nares. The term 'residence' within the nasopharynx is used to mean that the composition, once delivered to the nasopharynx, remains within the nasopharynx over a course of several hours, rather than being washed away more or less immediately. Preferred compositions include spray compositions and creams. Representative spray compositions include aqueous compositions, as well as oily compositions which contain amphiphilic agents so that the composition increases in viscosity when in contact with moisture. Creams may also be

used, especially creams having a rheology that allows the cream to spread readily in the nasopharynx.

Preferred aqueous spray compositions include, in addition to water, further excipients including a tonicity modifier such as a salt, for instance sodium chloride; preservative, such as benzalkonium salt; a surfactant such as a non-ionic surfactant, for instance a polysorbate; and buffer, such as sodium dihydrogen phosphate; present in low levels, typically less than 1%. The pH of the composition may also be adjusted, for optimum stability of the drug substance during storage. For compounds of the present invention, a pH in the range 5 to 6, preferably about 5.3 to 5.8, typically about 5.5 is optimal.

Representative oily spray and cream compositions are described in WO 98/14189 (SmithKline Beecham). Representative aqueous sprays are described in International Application no PCT/GB98/03211 (SmithKline Beecham).

Suitably, the drug substance is present in compositions for nasal delivery in between 0.001 and 5%, preferably 0.005 and 3%, by weight of the composition. Suitable amounts include 0.5% and 1% by weight of the composition (for oily compositions and creams) and from 0.01 to 0.2% (aqueous compositions).

Spray compositions according to the present invention may be delivered to the nasal cavity by spray devices well known in the art for nasal sprays, for instance an air lift pump. Preferred devices include those which are metered to provide a unit volume of composition, preferably about 100µl, and optionally adpated for nasal administration by addition of a modified nozzle.

The invention is illustrated by the following Examples.

Example 101 2,8-Diaza-8-methyl-1-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester

Step 1 Ethyl 1-*tert*-butyloxycarbonylpiperidine-4-carboxylate - Ethyl piperidine-4-carboxylate (10 g, 0.063 mole) in 1,2-dimethoxyethane (100 ml) and water (100 ml) at 0°C was treated with di-*tert*-butyldicarbonate (15.3 g, 0.07 mole) and triethylamine (9.7 ml, 0.07 mole) and then stirred at ambient temperature for 60 hours. Water (400 ml) was added and the product extracted into ethyl acetate (2 x 200 ml), dried (Na_2SO_4), filtered and evaporated to dryness to give the title compound as a colourless oil 17.9g (100%) ^1H NMR ($CDCl_3$) 1.25 (3H, t, J 7Hz), 1.45 (9H, s), 1.58-1.62(2H, m), 2.35-2.50(1H, m), 2.73-2.90 (2H, m), 3.95-4.08(2H, m), 4.15(2H, q, J 7Hz).

Step 2 Ethyl 1-*tert*-butyloxycarbonyl-4-(2-chloroethyl)-piperidine-4-carboxylate - Ethyl 1-*tert*-butyloxycarbonylpiperidine-4-carboxylate (10 g, 0.039 mole) in dry tetrahyrofuran (200 ml) at -60°C was treated with 2.0 molar lithium di-*iso*-propylamide (20 ml, 0.04 mole) and kept at -60°C for 2 hours. 1-Bromo-2-chloroethane (3.3ml, 0.04 mole) was added and the mixture allowed to warm to ambient temperature over 18 hours. Saturated ammonium chloride (100 ml) was added and the mixture extracted with diethyl ether (2 x 250 ml), washed with saturated brine, separated, dried (Na_2SO_4), filtered and evaporated to dryness to give the title compound as an oil 10.4 g (83%):M.S. (APCI) m/z 220 and 222 (MH^+-BOC-^{35}Cl, 100% and MH^+-BOC-^{37}Cl, 33%).

Step 3 Ethyl 1-*tert*-butyloxycarbonyl-4-(2-azidoethyl)-piperidine-4-carboxylate - Ethyl 1-*tert*-butyloxycarbonyl-4-(2-chloroethyl)-piperidine-4-carboxylate (3.5 g, 0.011 mole) in dimethylformamide (50 ml) was treated with sodium azide (1.0 g, 0.015 mole) and stirred at 90°C for 18 hours. The mixture was evaporated to dryness and the residue extracted with diethyl ether (50 ml) and washed with water (3x50 ml) dried (Na_2SO_4), filtered and evaporated to dryness to give the title compound 3.0 g (84%): M.S. (APCI) m/z 227 (MH^+-BOC, 100%).

Step 4 2,8-Diaza-8-*tert*-butyloxycarbonyl-1-oxospiro[4.5]decane - Ethyl *tert*-butyloxycarbonyl-4-(2-azidoethyl)-piperidine-4-carboxylate (3.0 g, 0.009 mole) was hydrogenated at 1 atmosphere in ethanol (50 ml) over 20% palladium on carbon (0.2 g) at 25°C for 18 hours. The catalyst was filtered off on kieselguhr and the filtrate concentrated *in vacuo*. Trituration of the residue with 60-80 petroleum ether gave the

title compound 0.72g (31%) as an off-white solid: M.S. (APCI) m/z 155 (MH^+-BOC, 100%).

Step 5 2,8-Diaza-8-methyl-1-oxospiro[4.5]decane – 2,8-Diaza-8-*tert*-butyloxycarbonyl-1-oxospiro[4.5]decane (1.3 g, 0.005 mole) in dichloromethane (80 ml) was treated with trifluoroacetic acid and heated under reflux for 4 hours. The mixture was evaporated to dryness and the residue partitioned between saturated potassium carbonate and 10% methanol/chloroform (2 x 50 ml). The organics were dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution (40:9:1) gave 2,8-diaza-1-oxospiro[4.5]decane. Treatment of this with 37% formaldehyde in water (0.3 ml) in ethanol (20 ml) and hydrogenation at 1 atmosphere over 10% palladium on carbon (0.05 g) for 18 hours. Filtration to remove the catalyst and evaporation of the filtrate to dryness gave the title compound 0.24 g (28%) ^1H NMR (DMSO-d^6) 1.21-1.31 (2H, d, J 13Hz), 1.60-1.75 (2H, m), 1.85-1.99 (4H, m), 2.15 (3H, s), 2.61-2.74 (2H, m), 3.15 (2H, t, J 7Hz).

Step 6 2,8-Diaza-8-methyl-1-oxospiro[4.5]decane-2-carboxylic acid (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin 14-ester - 2,8-Diaza-8-methyl-1-oxospiro[4.5]decane (0.24 g, 0.0014 mole) in tetrahydrofuran (15 ml) at -60 °C was treated with 2.0 molar lithium diisopropylamide (0.85 ml, 0.0017 mole) and stirred for 1 hour. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin-14-chloroformate (Example 12 of patent PCT/EP96/05874) (0.55g, 0.0014 mole) was then added and the mixture allowed to warm to ambient temperature over 18 hours. Water (50ml) was added and the product extracted into diethyl ether (2x100 ml). The organics were separated, dried (Na_2SO_4) filtered and evaporated to dryness. Chromatography on silica-gel eluting with chloroform/methanol/35% ammonia solution (95:4.5:0.5) gave the title compound as a foam 0.48 g (65%): M.S. (+ve ion electrospray) m/z 529 (MH^+, 100%).

Step 7 2,8-Diaza-8-methyl-1-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester - The title compound was prepared as in the method of Example 87, Step 4 of PCT/EP96/05874 from 2,8-Diaza-8-methyl-1-oxospiro[4.5]decane-2-carboxylic acid (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin 14-ester (0.48 g, 0.0009 mole) to give 0.34 g (73%) as a foam: M.S. (+ve ion electrospray) m/z 515 (MH^+, 100%).

Example 102 2,9-Diaza-9-methyl-1-oxospiro[5.5]undecane-2-carboxylic acid mutilin 14-ester - The title compound was prepared analogously to Example 101, Steps 1-7 except the alkylating agent used in step 2 was 1-bromo-3-chloropropane. This gave 0.085 g (32% final step): M.S. (+ve electrospray) m/z 529 (MH^+, 100%).

Example 103 2,4,8-Triaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester

Step 1 2,4,8-Triaza-8-methyl-1,3-dioxospiro[4.5]decane-2-carboxylic acid (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin 14-ester - 2,4,8-Triaza-8-methyl-1,3-dioxospiro[4.5]decane (see Coursison, J.C. *et al*, Farmaco, Ed. Sci (1988), 43 (2), 153-160) (0.1 g, 0.0005 mole) in dimethylformamide (2 ml) was treated with 60% sodium hydride in oil (0.025 g, 0.0006 mole) and stirred for 1 hour. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin-14-chloroformate (0.215 g, 0.005 mole) was added and the mixture stirred for 18 hours. The solvent was removed *in- vacuo* and the residue extracted into dichloromethane (50 ml) and washed with water (3 x 50 ml). The organic phase was separated, dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography on silica gel eluting with chloroform/methanol/35% aqueous ammonia (90:9:1) gave the title compound 0.085 g (29%): M.S. (+ve ion electrospray) m/z 544 (MH^+, 34%).

Step 2 2,4,8-Triaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester - The title compound was prepared as in Example 101, Step 7 from 2,4,8-Triaza-8-methyl-1,3-dioxospiro[4.5]decane-2-carboxylic acid (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin 14-ester. This gave 0.015 g (20%): M.S. (+ve ion electrospray) m/z 530 (MH^+, 25%).

Example 104 2,8-Diaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester - The title compound was prepared in 2 steps by analogy with Example 103, Steps 1-2 from -2,8-diaza-8-methyl-1,3-dioxospiro[4.5]decane [see Valenta *et al*, Collect. Czech. Chem. Commun. (1990), 55(9), 2304-2316] to give 0.016 g (4% 2 steps): M.S. (+ve ion electrospray) m/z 529 (MH^+, 100%).

Example 105 2,8-Diaza-8-methyl-3-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester - The title compound was prepared by analogy to Example 101, Steps 6-7 from 2,8-diaza-8-methyl-3-oxospiro[4.5]decane [see Cignarella, G. *et al*, J. Heterocylic Chem., (1993) 30 (5) 1357-1389]. This gave 0.084 g (27%, 2 steps). M.S. (+ve ion electrospray) m/z 515 (MH^+, 100%).

Example 106 2,8-Diaza-1-oxospiro [4.5]decane-2-carboxylic acid mutilin 14-ester

Step 1 2,8-Diaza-8-*tert*-butyloxyxcarbonyl-1-oxospiro[4.5]decane-2-carboxylic acid (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin 14-ester - The title compound was prepared as in the method of Example 101, Step 6 from 2,8-diaza-8-*tert*-butyloxcarbonyl-oxospiro[4.5]decane (2.0 g, 0.0079 mole) to give 3.4 g (70%); ^{1}H NMR ($CDCl_3$) *inter alia* 0.78 (3H ,d, J 7Hz), 0.95 (3H, d, J 7Hz), 1.20 (3H, s), 1.35 (3H, s), 1.42 (9H, s), 3.21 (2H, s) 3.75 (2H, t, J 7Hz), 4.95 (1H, d, J 17Hz), 5.28 (1H, d, J 11Hz), 5.75 (1H, d, J 11Hz), 6.73 (1H, dd, J 17 and 11Hz).

Step 2 2,8-Diaza-1-oxospiro [4.5]decane-2-carboxylic acid mutilin 14-ester

The title compound was prepared as in the method of Example 87, Step 4 of PCT/EP96/05874 from 2,8-Diaza-8-*tert*-butyloxyxcarbonyl-1-oxospiro[4.5]decane-2-carboxylic acid (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*mutilin 14-ester (3.4 g, 0.0055 mole). This procedure also removes the *tert*-butyloxycarbonyl group to give 2.4 g (87%) M.S. (+ve ion electrospray) m/z 501 (MH^+, 100%).

Example 107 2,8-Diaza-8-*tert*-butyloxycarbonylmethyl-1-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester - 2,8-Diaza-8-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester (Example 106) (1.2 g, 0.0024 mole) in dimethylformamide (20 ml) was treated with potassium carbonate (1.0 g, 0.0072 mole) and *tert*-butyl bromoacetate (0.43 ml, 0.0029 mole) and stirred at ambient temperature for 18 hours. The mixture was evaporated to dryness and the residue extracted with ethyl acetate (50 ml) and washed with water (3 x 50 ml), separated, dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography on silica gel eluting with dichloromethane/methanol/35% ammonia solution (10:0.23:0.025) gave 1.0 g (68%): M.S. (+ve ion electrospray) m/z 615 (MH^+, 55%).

Example 108 (3*R*,4*S*)-Spiro[(1-azabicyclo[2,2,1]heptane-3,3'-(2'-oxo-pyrrolidine)]-1'-carboxylic acid mutilin 14-ester - The title compound was prepared as in the method of Example 101, Steps 2-4 and 6-7 from ethyl (3*R*,4*S*)-1-azabicyclo[2,2,1]heptane-3-carboxylate (see Cottrell D. *et al,* J.Chem. Soc. *Perkin Trans* 1 (1991), 1091-1097) to give 0.16 g (74%, final step): M.S. (+ve ion electrospray) m/z 513 (MH+, 60%).

Example 109 (3*R*,4*S*)-Spiro[(1-azabicyclo[2,2,1]-heptane)-3,3'-(2'-oxopyrrolidine)]-1'-carboxylic acid (2*S*)-2-hydroxy-mutilin 14-ester

Step 1 Formylated derivatives of mutilin - The reaction was carried out similarly to that described by A.J. Birch, C.W. Holzapfel and R.W. Rickards (Tet (Suppl) 1996 8 part III 359). Mutilin (6 g) in toluene (330 ml) and methyl formate (100 ml) was treated with sodium methoxide (3 g) and stirred under argon for 8 hours. Ice-water (100 ml) was added, followed by 2N HCl (220 ml). The mixture was shaken and separated and the aqueous extracted with ether. The combined organic was dried and evaporated and the residue chromatographed, eluting with ethyl acetate/hexane mixtures. First eluted was 2-hydroxymethylene mutilin 11,14-diformate (2.33 g) ^{1}HNMR ($CDCl_3$) *inter alia* 5.02 (1H, d), 5.77 (1H, d), 6.94 (1H, s), 7.89 (1H, s), 8.10 (1H, s). Second to be eluted was 2-hydroxymethylene-mutilin 11-formate (3.0 g): ^{1}H NMR ($CDCl_3$) *inter alia* 4.40 (1H, d), 5.11 (1H, d), 7.06 (1H, s), 8.25 (1H, d, J O.8Hz). Third to be eluted was a mixture (2:1) of 2-hydroxymethylene-mutilin 14-formate and 2-hydroxymethylene-mutilin (1.8 g).

Step 2 2-Hydroxymethylene-mutilin - A mixture of 2-hydroxymethylene-mutilin 11,14-diformate (2.33 g) and [2-hydroxymethylene-mutilin 14-formate + 2-hydroxymethylene-mutilin] (18 g) was dissolved in ethanol (30 ml) and treated with 0.5M KOH in ethanol (60 ml). After 1 hour the solution was diluted with ethyl acetate (200 ml), washed with 2M HCl (120 ml) and water (100 ml), dried and evaporated to provide 2-hydroxymethylene-mutilin as a foam (3.6 g); ^{1}H NMR ($CDCl_3$) *inter alia* 3.45 (1H, d), 4.37 (1H, d), 6.97 (1H, s).

Step 3 2-Diazomutilin - A solution of 2-hydroxymethylenemutilin (3.6 g) in dichloromethane was cooled to -10°C under argon, treated with triethylamine (4.6 ml) and tosyl azide (3.55 g) and warmed to room temperature. After 6 hours the solution was washed with 0.5M HCl (150 ml) and water (100 ml), dried and evaporated. The 2-

diazomutilin was obtained as yellow crystals (1.7 g) from ethyl acetate/hexane; IR ($CHCl_3$) 3634, 2082 and 1670 cm^{-1}.

Step 4 (2*S*)-2-Dichloroacetoxy-mutilin - A solution of 2-diazomutilin (1.7 g) in dichloromethane (40 ml) was ice-cooled and treated dropwise with dichloracetic acid (0.5 ml). The bath was removed and after 30 minutes the solution was colourless. It was washed with aqueous $NaHCO_3$ (50 ml), dried and evaporated. Chromatography eluting with 1:3 ethyl acetate/hexane, gave the title compound as the less polar of 2 major products (white foam, 1.6 g): ^{1}H NMR ($CDCl_3$) *inter alia* 3.33 (1H, t, J 5.8Hz), 4.33 (1H, d, J 7Hz), 5.04 (1H, t, J 9Hz), 5.2-5.4 (2H, m), 5.96 (1H, s), 6.14 (1H, dd, J 17.5 and 10.5 Hz).

Step 5 (2*S*)-2-Dichloroacetoxy-11-*O*-trifluoroacetyl-mutilin - (2*S*)-2-Dichloroacetoxymutilin (5.8 g, 0.012 mole) in dry tetrahydrofuran (120 ml) was treated with trifluoroacetylimidazole (1.54 ml, 0.0135 mole) and stirred at ambient temperature for 18 hours. Ethyl acetate (200 ml) was added to the mixture which was then washed with dilute sodium chloride solution (2 x 200 ml). The organic layer was separated, dried (Na_2SO_4), filtered and evaporated to dryness. Chromatography on silica gel eluting with ethylacetate/hexane (9:1) gave the title compound 4.98 g (71%) ^{1}H NMR ($CDCl_3$) *inter alia* 0.85 (3H, d, J 7Hz), 0.95 (3H, d, J 7Hz), 1.05 (3H, s), 1.39 (3H, s), 4.29 (1H, t, J 7Hz), 4.86 (1H, d, J 7Hz), 5.08 (1H, t, J 9Hz), 5.99 (1H, s).

Step 6 (2*S*)-2-Dichloroacetoxy-11-*O*-trifluoroacetyl-mutilin 14-chloroformate - The title compound was prepared as in the method of Example 12 of patent PCT/EP96/05874 from (2S)-2-dichloroacetoxy-11-*O*-trifluoroacetyl-mutilin to give 1.16 g (100%). ^{1}H NMR ($CDCl_3$) *inter alia* 0.85 (3H, d, J 7Hz), 0.87 (3H, d, J 7Hz), 1.13 (3H, s), 1.51 (3H, s), 4.85 (1H, d, J 7Hz), 5.05 (1H, t, J 9Hz), 5.95 (1H, s).

Step 7 (3*R*,4*S*)-Spiro[(1-azabicyclo[2,2,1]-heptane)-3,3'-(2'-oxopyrrolidine)]-1'-carboxylic acid (2*S*)-2-hydroxy-mutilin 14-ester - The title compound was prepared from (3*R*,4*S*)-spiro[(1-azabicyclo[2,2,1]heptane)-3,3'-(2'-oxopyrrolidine) (see Example 108 and Example 101, step 6 for details) and (2*S*)-2-dichloroacetoxy-11-*O*-trifluoroacetyl-mutilin 14-chloroformate. Careful hydrolysis of the mutilin 2-dichloroacetoxy and 11-trifluoroacetoxy groups with 2 equivalents of potassium hydroxide in ethanol followed by chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution (10:0.23:0.025) followed by trituration with

methanol/diethyl ether gave the title compound 0.039 g (11%, 2 steps). M.S. (+ve ion electrospray) m/z 529 (MH^+, 85%).

Example 201 Mutilin 14-(4-nitrophenyl) acetate - Mutilin (0.32 g, 0.001 mole) was dissolved in dichloromethane (8 ml) and (4-nitrophenyl) acetyl chloride (0.239 g, 0.0012 mole) and pyridine (0.097 ml 0.0012 mole) were added. The resulting mixture was stirred under an argon atmosphere. After 1 hour additional (4-nitrophenyl) acetyl chloride (0.06 g, 0.0003 mole) and pyridine (0.024 ml, 0.0003 mole) were added. After stirring for a further 1 hour the mixture was concentrated *in vacuo*; the residue was partitioned between ethyl acetate and water. The ethyl acetate solution was separated, washed with water, aqueous sodium hydrogen carbonate solution and brine, dried over magnesium sulphate and concentrated *in vacuo*. Silica gel chromatography eluting with acetone/toluene mixtures gave the title compound (0.179 g, 37%); MS (electron ionisation) m/z 483 (M^+).

Example 202 Mutilin 14-phenylacetate - The title compound (0.258 g, 19%) was prepared using the method of Example 201; MS (electron ionisation) m/z 438 (M^+).

Example 203 Mutilin 14-(2-thienyl) acetate

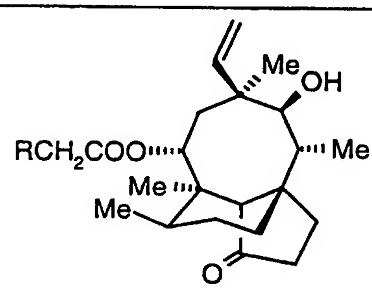
Step 1. (2- Thienyl) acetyl chloride - (2-Thienyl) acetic acid (0.355 g, 0.0025 mole) was dissolved in dichloromethane (20 ml) and N,N-dimethylformamide (0.05 ml) and oxalyl chloride (0.44 ml) were added. The resulting solution was stirred under an argon atmosphere for 4 hours. Following concentration *in vacuo* the residue was taken up in a mixture of dichloromethane and toluene, concentrated *in vacuo* then dried *in vacuo* (0.25 mmHg, 15 minutes) to give the title compound.

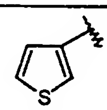
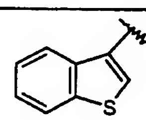
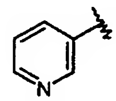
Step 2. Mutilin 14-(2-thienyl) acetate-11-trifluoroacetate - Mutilin 11-trifluoroacetate (0.625 g, 0.0015 mole) and (2-thienyl) acetyl chloride (0.0025 mole theoretical, Step 1) were dissolved in N,N-dimethylformamide (5 ml) and the resulting solution heated at 110°C for 17 hours under an argon atmosphere. The reaction mixture was then diluted with ethyl acetate (100 ml) and washed with water (3 x 50 ml), saturated aqueous sodium hydrogen carbonate (1 x 50 ml) and brine (1 x 50 ml). Drying over magnesium sulfate was followed by concentration *in vacuo*. The resulting residue was chromotographed on

silica gel eluting with ethyl acetate/hexane mixtures to give the title compound (0.41 g, 50%); MS (-ve ion electrospray) m/z 539 ([M-H]$^-$).

Step 3. Mutilin 14-(2-thienyl) acetate - Mutilin 14-(2-thienyl) acetate-11-trifluoroacetate (0.41 g, 0.00076 mole) was dissolved in ethanol (20 ml) and the solution cooled in an ice bath. 0.5M Potassium hydroxide in ethanol (1.5 ml) was then added dropwise. After 5 minutes the mixture was concentrated *in vacuo* and the residue taken up in ethyl acetate, washed with water, saturated aqueous sodium hydrogen carbonate solution and brine, then dried over magnesium sulphate. Concentration *in vacuo* gave the crude product which was chromotographed on silica gel eluting with ethyl acetate/hexane mixtures to give the title compound (0.31 g, 91%); MS (-ve ion electrospray) m/z 443 ([M-H]$^-$).

The following compounds were also prepared using the procedure of Example 203.



Example	R	M.S.
204		m/z 443 ([M-H]$^-$)
205		m/z 493 ([M-H]$^-$)
206		m/z 438 ([M-H]$^-$)

Example 207 Mutilin 14-[4-(1-Methylpiperidin-4-ylmethyloxy)] phenylacetate

Step 1. Methyl 4-(1-methylpiperidin-4-ylmethyloxy) phenylacetate - Triphenylphosphine (2.23 g, 0.0085 mole) in dry tetrahydrofuran was cooled to 0°C and treated dropwise with diethylazodicarboxylate (1.34 ml, 0.0085 mole) and the mixture stirred for 30 minutes. 1-Methylpiperidine-4-methanol (1.0 g, 0.0078 mole) and methyl 4-hydroxyphenylacetate (1.30 g, 0.00085 mole) in dry tetrahydrofuran were added dropwise to the mixture and stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue partitioned between 1M hydrochloric acid and diethyl ether. The aqueous layer was washed with diethyl ether, basified with potassium carbonate and extracted with dichloromethane. The organic extract was then dried over magnesium sulphate and concentrated *in vacuo* to afford the title compound (1.19g, 55%) as a yellow oil; MS (+ve ion electrospray) m/z 278 (MH^+).

Step 2. 4-(1-Methylpiperidin-4-ylmethyloxy) phenylacetic acid hydrochloride - The product of Step 1 (1.1 g, 0.004 mole) in concentrated hydrochloric acid (10 ml) was heated under reflux overnight. The solvent was removed *in vacuo*, the residue was azeotroped with toluene to afford the title compound (1.2 g, 100%) as a cream solid; MS (+ve ion electrospray) m/z 264 (MH^+).

Step 3. 4-(1-Methylpiperidin-4-ylmethyloxy) phenylacetyl chloride - The product of Step 2 (0.30 g, 0.0014 mole) in dry dichloromethane was treated with oxalyl chloride (0.3 ml, 0.0034 mole) and DMF (0.05 ml). The mixture was stirred at room temperature for 4.5 hours. The solvent was removed *in vacuo*, and the residue azeotroped with toluene to afford the title compound (0.36g, 100%). MS (+ve ion electrospray in methanol) m/z 278 (MH^+ methyl ester).

Step 4. (3R)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14[4-(1 methylpiperidin-4-ylmethyloxy)] phenylacetate - The product of Step 3 (0.36 g, 0.0011 mole) in dry N,N-dimethylformamide was treated with (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin (0.38 g, 0.0011 mole) and heated at 110°C overnight. The solvent was removed *in vacuo* and the residue purified by column chromatography, eluting with 1-5% methanol/dichloromethane to afford the title compound (0.13g, 20%) as a yellow solid; MS (+ve ion electrospray) m/z 580 (MH^+).

Step 5. Mutilin 14-[4-(1-methylpiperidin-4-ylmethyloxy)] phenylacetate - The product from Step 4 (0.11 g, 0.00022 mole) in 1,4-dioxane (2 ml) and concentrated hydrochloric acid (3 ml) was stirred at room temperature for 3 hours. The mixture was

diluted with water, basified with sodium hydrogen carbonate, extracted into dichloromethane, dried over magnesium sulphate and evaporated in *vacuo*. The residue was purified by column chromatography eluting with 2% of a 9:1 methanol/35% aqueous ammonia solution in dichloromethane to afford the title compound (0.05g, 40%) as a colourless solid; MS (+ve ion electrospray) m/z 566 (MH^+).

Example 208 Mutilin 14-(2-methylthiazol-4-yl) acetate

Step 1. (3R)-3-Deoxo-3-methoxy-11-oxo-4-*epi*-mutilin 14-(4-chloro-2-oxo-butyrate) - A solution of (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin (5.0 g, 0.015 mole) in toluene (120 ml) was treated with ethyl 4-chloroacetoacetate (4.0 ml, 0.03 mole) and 4-dimethylaminopyridine (0.90 g, 0.0075 mole) and the mixture was heated under reflux for 72 hours. The mixture was then partitioned between ethyl acetate and aqueous ammonium chloride solution. The organic extracts were dried over magnesium sulphate and evaporated *in vacuo*. The residue was purified by column chromatography eluting with 1-2% ethyl acetate in dichloromethane to afford the title compound (3.39g, 50%) as a yellow oil; MS (-ve ion electrospray) m/z 451 $(M-H)^-$.

Step 2. (3R)-3-Deoxo-3-methoxy-11-oxo-4-*epi*-mutilin 14-(2-methylthiazol-4-yl) acetate - The product of Step 1 (3.4 g, 0.0075 mole), and thioacetamide (0.56 g, 0.0075 mole) were heated in ethanol under reflux for 3.5 hours. The reaction mixture was diluted with water and extracted into ethyl acetate. The ethyl acetate layer was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by column chromatography to afford the title compound; ^1H NMR ($CDCl_3$) *inter alia* 0.73(3H, d, J 7Hz), 0.99(3H, d, J 7Hz), 2.70(3H, s), 3.22(3H, s), 3.77(2H, s), 5.00(1H, d, J 17Hz), 5.28(1H, d, J 13Hz), 5.75(1H, d, J 10Hz), 6.68(1H, dd, J 17, 10Hz), 7.00(1H, s).

Step 3. Mutilin 14-(2-methyl thiazol-4-yl) acetate - The title compound was prepared from the product of Step 2 (0.5 g, 0.001 mole) using the method of Example 7 Step 5; MS (+ve ion electrospray) m/z 460 (MH^+).

Example 209 Mutilin 14-(2-dimethylaminomethylfur-3-yl) acetate

Step 1. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(fur-3-yl)acetate - Trimethylsilyl-diazomethane (18.75 ml, 0.038 mole) and triethylamine (4.3 ml, 0.031

mole) in 1:1 THF/acetonitrile (60ml) was cooled to 0°C and treated dropwise with 3-furoyl chloride (K. Kuhn, *Chem. Ber.*, 1956, **89**, 1473: 3.25g, 0.025 mole). The mixture was kept at 0°C for 48 hours. The solvent was removed in vacuo the residue dissolved in 2,4,6-trimethylpyridine (20ml) and treated with (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin (8.0g, 0.025 mole). The mixture was then heated to 180°C for 10 minutes and allowed to cool to room temperature. The reaction mixture was diluted with ethyl acetate, washed with brine, dried ($MgSO_4$), and concentrated *in vacuo*. The product was purified by column chromatography, eluting with 5-10% ethyl acetate/hexane, to afford the title compound (1.07g, 10%) as a yellow oil: ^{1}H NMR ($CDCl_3$) *inter alia* 7.41 (1H, s), 7.40 (1H, s), 6.66 (1H, dd, J 17, 10Hz), 6.35 (1H, m), 6.22 (1H, m), 5.74 (1H, d, J 10Hz), 5.28 (1H, d, J 10Hz), 4.99 (1H, d, J 17Hz), 3.66 (2H, s), 3.44 (1H, m), 3.22 (3H, s), 2.91 (1H, m), 2.44 (1H, m), 2.19 (1H, m), 0.97 (3H, d, J 7Hz), 0.73 (3H, d, J 7Hz).

*Step 2. (3R)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(2-dimethyl aminomethylfur-3-yl)acetate, and (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(5-dimethylaminomethylfur-3-yl)acetate* - Glacial acetic (15 ml) was cooled in an ice bath and treated with 40% aqueous dimethylamine (0.32 ml, 0.0029 mole) and 37% aq. formaldehyde (0.23 ml, 0.0029 mole). This mixture was then added to the product of Step 1 (1.05g, 0.0024 mole) with stirring. The reaction mixture was briefly warmed in a water bath, stirred at room temperature for 0.5 hours then and heated at 100°C for 50 minutes. The reaction mixture was diluted with 2M sodium hydroxide solution and extracted with ethyl acetate. The ethyl acetate layer was washed with water, brine, dried ($MgSO_4$), and concentrated *in vacuo*. The product was purified by column chromatography, eluting with 2-10% methanol in dichloromethane. (3R)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(2-dimethylaminomethylfur-3-yl)acetate (Product A, 0.28g, 23%) was found to be the less polar compound: MS (+ve ion electrospray) m/z 500 (MH+). (3R)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(5-dimethyl aminomethylfur-3-yl)acetate (Product B, 0.03g, 2.5%) was found to be the more polar compound: MS (+ve ion electrospray) m/z 500 (MH+).

Step 3 Mutilin 14-(2-dimethylaminoethylfur-3-yl)acetate - The title compound (0.20g, 76%) was obtained from Product A from Step 2 by the method described in Example 207 Step 5: MS (+ve ion electrospray) m/z 486 (MH^+).

Example 210 Mutilin 14-(5-dimethylaminoethylfur-3-yl)acetate - The title compound was prepared from Example 9, Step 2, Product B using the method described in Example 207 Step 5; MS (+ve ion electrospray) m/z 486 (MH+).

Example 211 Mutilin 14-(2-furyl) acetate

Step 1. (3R)-3-Deoxo-3-methoxy-11-oxo-4-*epi*-mutilin 14-(2-furyl) acetate - The title compound was prepared from 2-furoyl chloride and (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin, using the method described in Example 209, Step 1; MS (-ve ion electrospray) m/z 441 ($[M-H]^-$).

Step 2. Mutilin 14-(2-furyl) acetate - The title compound was prepared from the product of Step 1 using the method of Example 7, Step 5; ^{1}HNMR ($CDCl_3$) *inter alia* 0.56(3H, d, J 7Hz), 0.88(3H, d, J 7Hz), 3.60 (2H, s), 5.20 (1H, d, J 17Hz), 5.35 (1H, d, J 11Hz), 5.74(1H, d, J 8 Hz), 6.19 (1H, m), 6.51(1H, dd, J 17, 11Hz), 7.38 (1H, s).

Example 212 Mutilin 14-(5-Dimethylaminomethyl-2-furyl) acetate - The title compound (0.01g, 16%) was prepared from Example 211 (0.056g, 0.00013mole) using the method described in Example 9, Step 2: MS (+ve ion electrospray) m/z 486 (MH^+).

Example 213 Mutilin 14-(3-methylisoxazole-5-yl) acetate

Step 1. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(3-methylisoxazole-5-yl) acetate

A solution of 3,5-dimethylisoxazole (0.194 g, 0.002 mole) in tetrahydrofuran (5 ml) at -78°C under argon was treated over 15 minutes with *n*-butyllithium (1.6M in hexanes, 1.25 ml). A solution of (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-chloroformate (0.793 g, 0.002 mole) in tetrahydrofuran (2 ml) was added dropwise and the solution was stirred at -78°C for 1 hour. The reaction was allowed to warm up to -50°C and saturated ammonium chloride solution (2 ml) was added. The mixture was diluted with ethyl acetate and dilute hydrochloric acid was then added to adjust the pH to 5. The organic phase was separated, washed with water and brine, dried over magnesium sulphate and the solvent removed in vacuo. Chromatography of the residue on silica gel

eluting with 10% ethyl acetate in hexanes gave the title compound (0.213g, 23%); MS (ammonia CI) m/z 458 (MH^+), 475 ($[M+NH_4]^+$).

Step 2. Mutilin 14-(3-methylisoxazole-5-yl) acetate - The title compound (0.11g, 57%) was prepared from the product of Step 1, using the method described in Example 207, Step 5: MS (ammonia CI) m/z 444 (MH^+), 461 ($[M+NH_4]^+$).

Example 214 Mutilin 14-[2-(piperidin-4-yl)thiazol-4-yl]acetate

Step 1. N-*tert*-Butoxycarbonylpiperidine-4-carboxamide - Piperidine-4-carboxamide (2.5g, 0.020 mole) in dichloromethane was treated with di-*tert*-butyl dicarbonate (6.36g, 0.030 mole) and stirred at room temperature for 16 hours. The reaction mixture was washed with water, and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by column chromatography, eluting with 1-4% methanol in dichloromethane to afford the title compound (3.3g, 72%) as a white solid. MS (+ve ion electrospray) m/z 229 (MH+).

Step 2. N-*tert*-butoxycarbonylpiperidine-4-thiocarboxamide - The product of Step 1 (0.5g, 0.002 mole) in dichloromethane was treated with Lawesson's reagent (0.44g, 0.001 mole) and stirred at room temperature for 16 hours. The solvent was removed *in vacuo* to afford the title compound (0.36g, 74%) as a yellow solid; MS (+ve ion electrospray) m/z 245 (MH+).

Step 3. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[2-(piperidin-4-yl)thiazol-4-yl] acetate - The product of Step 2 (0.35g, 0.0015 mole) and (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-chloro-2-oxo-butyrate (Example 8, Step 1) (0.694g, 0.0015 mole) in ethanol were heated under reflux for 3.5 hours. The reaction mixture was diluted with water and extracted into ethyl acetate. The ethyl acetate layer was washed with water and brine, dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified by column chromatography, eluting with 1-2% ethyl acetate in dichloromethane to afford the title compound (0.097g, 12%) as a yellow solid; MS (+ve ion electrospray) m/z 543 (MH+).

Step 4. Mutilin 14-[2-(piperidin-4-yl)thiazol-4-yl] acetate - The the title compound (0.04g, 47%) was prepared from the product of Step 3 (0.097g, 0.17m mol) as described in Example 207, Step 5; 1H NMR (CDCl3) *inter alia* 7.00 (1H, s), 6.51 (1H, dd, J 17,

11Hz), 5.73 (1H, d, J 8Hz), 5.34 (1H, d, J 11Hz), 5.20 (1H, d, J 17Hz), 3.33 (1H, m), 3.30 (3H, m), 2.78 (2H, m), 0.86 (3H, d, J 7Hz), 0.60 (3H, d, J 7Hz).

Example 215 Mutilin 14-(4-dimethylaminomethylphenyl) acetate hydrochloride

Step 1. (4-Dimethylaminomethylphenyl)acetyl chloride - (4-Dimethylaminomethylphenyl) acetic acid (Zaugg *et al*, *J. Am. Chem. Soc*, 1958, **80**, 4317) (0.190g, 0.001mol) in dry dichloromethane was treated with oxalyl chloride (0.26ml, 0.003mol) and DMF (0.05ml). The mixture was stirred at room temperature for 2 hours. The solvent was removed *in vacuo*, and the residue azeotroped with toluene to afford the title compound (0.21g, 100%); MS (+ve ion electrospray in methanol) m/z 208 (MH^+ methyl ester).

Step 2. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(4-dimethylaminomethylphenyl) acetate - The product of Step 1 (0.21g, 0.001mol) in dry N,N-dimethylformamide was treated with (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin (0.267g, 0.0008mmol) and heated at 120°C overnight. The solvent was removed *in vacuo* and the residue dissolved in dichloromethane, washed with aqueous sodium bicarbonate, dried over magnesium sulphate and evaporated *in vacuo*. The residue was purified by column chromatography, eluting with 2-10% methanol in dichloromethane to afford the title compound (0.195g, 48%) as a colorless oil; MS (+ve ion electrospray) m/z 510 (MH^+).

Step 3. Mutilin 14-(4-dimethylaminomethylphenyl) acetate - The title compound (0.11g, 63%) was prepared from the product of Step 2 using the method of Example 207 Step 5; ^1H NMR ($CDCl_3$): *inter alia* 0.54 (3H, d, J 6.4Hz), 0.85 (3H, d, J 6.9Hz), 2.22 (6H, s), 3.35 (1H, m), 3.39 (2H, s), 3.53 (2H, s), 5.16 (1H, d, J 17.5Hz), 5.33 (1H, d, J 10.9Hz),5.70 (1H, d, J 8.5Hz), 6.49 (1H, dd, J 17.4Hz, 11.0Hz), 7.21 (4H, m).

Example 216Mutilin 14-(2-cyanomethylthiazol-4-yl)-acetate

Step 1. (3R)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14(2-cyanomethyl-thiazol-4-yl) acetate - The title compound (1.3g, 39%) was prepared from 2-cyanothioacetamide (0.665g, 0.0066mol) and (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-

4-*epi*-mutilin 14-chloro acetoacetate (Example 208, Step 1) (6.0g, 13.3mmol) using the method described in Example 214 Step 3; MS (-ve ion electrospray) m/z 497 ([M-H]$^-$).

Step 2. Mutilin 14-(2-cyanomethyl-thiazol-4-yl) acetate - The the title compound (0.91g, 73%) was obtained from the product of Step 1 using the method described in Example 207 Step 5; MS (-ve ion electrospray) m/z 483 ([M-H]$^-$).

Example 217 Mutilin 14-(2-carbamoylmethylthiazol-4-yl)-acetate - Hydrogen chloride gas was bubbled through a stirred solution of the product from Example 16 (0.4g, 0.00083mol) and methanol (0.2ml) in dioxane (5ml). After 1 hour the solvent was evaporated and the residue triturated with diethyl ether giving a solid. The solid was dissolved in methanol (10ml) and ammonia gas was bubbled through the solution for 30 minutes. The mixture was then heated at 60°C for 16 hours under argon. After cooling to room temperature the solvent was evaporated and the residue purified by column chromatography on silica gel eluting with 2-5% of a 9:1 methanol/ 35% ammonia solution in dichloromethane to afford the title compound (230mg, 55%) as a yellow solid; MS (+ve ion electrospray) m/z 503 (MH$^+$).

Example 218 Mutilin 14-(4-carbamoylphenyl) acetate

Step 1. (3R)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(4- cyanophenyl) acetate - The title compound (350mg, 15%) was prepared from 4-cyanobenzoyl chloride and (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin using the method described in Example 209, Step 1; MS (-ve ion electrospray) m/z 476 ([M-H]$^-$).

Step 2. Mutilin 14-(4-carbamoyl-phenyl)-acetate - The title compound (50mg, 17%) was prepared from the product of Step 1 using the method described in Example 17; MS (+ve ion electrospray) m/z 482 (MH$^+$).

Also isolated from this reaction was a mixture of (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(4-carbamimidoylphenyl) acetate and mutilin 14-(4-carbamimidoyl phenyl) acetate.

Example 219 Mutilin 14-(4- carbamimidoyl-phenyl) acetate - The title compound was obtained from the mixture of (3R)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(4-carbamimidoyl-phenyl) acetate and mutilin 14-(4-carbamimidoyl-phenyl) acetate,

isolated from Example 18 Step 2, by treatment with concentrated hydrochloric acid in 1,4-dioxane as described in Example 207, Step 5; MS (+ve ion electrospray) m/z 481 (MH^+).

Example 220 Mutilin 14-[4-(2-dimethylaminoethyl)-carbamoyl-phenyl]-acetate

Step 1. Mutilin 14-(4-methoxycarbonylphenyl) acetate-11-trifluoroacetate - The title compound was prepared from methyl 4-chlorocarbonyl benzoate and mutilin-11-trifluoroacetate by analogy to the method described in Example 209 Step 1; ^{1}H NMR ($CDCl_3$): *inter alia* 0.57 (3H, d, J 6.6Hz), 0.81 (3H, d, J 7.0Hz), 3.60 (2H, s), 3.91 (3H, s), 4.96 (1H, d, J 6.8Hz), 5.15 (1H, d, J 17.6Hz), 5.28 (1H, d, J 11.3Hz), 5.65 (1H, d, J 8.1Hz), 6.32 (1H, dd, J 17.5, 11.3Hz), 7.32 (2H, d, J 8.2Hz), 8.00 (2H, d, J 8.2Hz).

Step 2. Mutilin 14-(4-carboxyphenyl) acetate - The product from Step 1 (4.2g, 7mmol) was dissolved in 1,4-dioxane (20ml), 2M aqueous sodium hydroxide (10ml) added and the mixture stirred at room temperature overnight. The dioxane was evaporated and the aqueous layer washed with ethyl acetate. The aqueous layer was then acidified with 2M hydrochloric acid and extracted with ethyl acetate. The organic extract was washed with brine, dried over magnesium sulphate and evaporated to afford the title compound as a white solid: MS (-ve ion electrospray) m/z 481 $(M-H)^-$.

Step 3. Mutilin 14-[4-(2-dimethylaminoethyl)-carbamoyl-phenyl]-acetate - The product from Step 2 (180mg, 0.37mmol) was dissolved in THF (3ml), 1,3-diisopropylcarbodiimide (0.06ml, 0.37mmol) was added, followed by a catalic amount of 1-hydroxybenzotriazole and N,N'-dimethylethylenediamine (0.04ml, 0.37mmol) and the mixture stirred at room temperature overnight. The reaction mixture was diluted with ethylacetate, washed with saturated aqueous sodium hydrogen carbonate and brine, dried over magnesium sulphate and evaporated. The residue was purified by column chromatography eluting with 2 to 7% of a 9:1 methanol/35% ammonia solution in dichloromethane to afford the title compound (70mg, 35%) as a white solid; MS (+ve ion electrospray) m/z 553 (MH^+).

Example 221 Mutilin 14-{4-[(2-dimethylaminoethyl)-methyl-carbamoyl]phenyl}-acetate - The title compound (65mg, 30%) was prepared from the product of Example

220, Step 2 and N,N,N'-trimethylethylendiamine using the method described in Example 220, Step 3; MS (+ve ion electrospray) m/z 567 (MH^+).

Example 222 Mutilin 14-{4-[4-(3-hydroxypropyl)-piperazine-1-carbonyl]-phenyl}-acetate - The title compound was prepared from the product of Example 220, Step 2 and 1-(3-hydroxypropyl)piperazine using the method of Example 220, Step 3; MS (+ve ion electrospray) m/z 609 (MH^+).

Example 223 Mutilin-14-[3-(piperazin-1-ylmethyl)-isoxazol-5-yl]-acetate

Step 1. 5-Methyl-3-(piperazinylmethyl) isoxazole - 5-Chloromethyl-3-methyl-isoxazole (500 mg, 3.8 mmol) (Jones et. al. *J. Org. Chem.*, 1954, **19**, 1428-1430) and piperazine (1.63 g, 19 mmol) were dissolved in toluene (15 ml) and heated at 70 °C for 1 hour. The mixture was cooled and partitioned betweem chloroform and saturated aqueous potassium carbonate. The organic fraction was dried (sodium sulfate), filtered and the solvent removed *in vacuo*. The residue was purified on silica gel eluting with dichloromethane/ methanol/ 35% ammonia solution (85:14:1) to afford the title compound (185 mg, 27%) as a crystalline solid; MS (+ve ion, CI) *m/z*:182 $(MH)^+$

Step 2. 3-(4-*tert*-Butoxycarbonyl-piperazin-1-yl-methyl)-5-methyl isoxazole - The product from step 1 (180 mg, 0.99 mmol) and di-*tert*-butyl dicarbonate (327 mg 1.5 mmol) were dissolved in dichloromethane (15 ml) and stirred for 48 hours. The solvent was removed and the residue purified on silica gel eluting with ethyl acetate : hexane (4:1) to afford the title compound (150 mg, 54%) as a crystalline solid. MS (+ve ion, CI) *m/z*: 282 $(MH)^+$.

Step 3. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[3-(4-*tert*-butoxycarbonyl-piperazin-1-ylmethyl)-isoxazol-5-yl]-acetate - The product from Step 2 was dissoved in tetrahydrofuran (4 ml) and cooled to -78°C under argon. n-Butyl lithium (1.53 M in hexane, 0.56 mmol, 0.365 ml) was added and the mixture stirred for 20 minutes. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-chloroformate (333 mg, 0.84 mmol) was then added dropwise as a solution in tetrahydrofuran (4 ml). The mixture was stirred for 1 hour at -78 °C and for a further hour at -50 °C. The reaction was then quenched with saturated aqueous ammonium chloride (1 ml) and then

the mixture partitioned between saturated aqueous potassium carbonate and chloroform. The organic layer was dried (sodium sulfate), filtered, the solvent removed and the residue purified on silca gel eluting with ethyl acetate:hexane (2:1) to afford the title compound (90 mg, 25%) as a yellow oil. MS (+ve ion electrospray) *m/z*: 642 $(M + H)^+$.

Step 4. Mutilin-14-[3-(piperazin-1-ylmethyl)-isoxazol-5-yl] acetate - The title compound (39 mg, 63%) was prepared from the product from Step3 (90 mg, 0.14 mmol) by the method described in Example 7, Step 5; MS (+ve ion electrospray) *m/z*: 528 $(M + H)^+$.

Reagent Preparations for Examples 301 to 349

Reagent 1 - 4-[(3-*exo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yloxy] phenol

Step 1. (3-*endo*)-3-Methanesulphonyloxy-8-methyl-8-aza-bicyclo[3.2.1]octane - To a solution of tropine (2.0 g, 14.2 mmol) in dichloromethane (100 ml) was added methanesulphonyl chloride (1.2 ml, 15.5 mmol) and triethylamine (2.2 ml, 15.8 mmol) at 0^oC. The reaction mixture was stirred at 0^0C for 1 hour and at room temperature for 3 hours. The mixture was washed with saturated aqueous sodium hydrogen carbonate, dried over magnesium sulphate and concentrated *in vacuo* to give the title compound (2.60g, 84%): MS (+ve ion electrospray) m/z 220 (MH^+).

Step 2. (3-*exo*) 3-(4-benzoylphenyloxy)-8-methyl-8-aza-bicyclo[3.2.1]octane - Hydroxyphenyl benzoate (1.0 g, 4.67 mmol) was dissolved in DMF (10 ml), treated with sodium hydride (182 mg, 4.55 mmol) and the mixture stirred for 30 minutes. The reaction was then cooled to 0^0C and the product from Step 1 (1.0 g, 4.57 mmol) was added. The mixture was stirred at 0^oC for 1 hour and then at room temperature for 14 hours. The reaction mixture was diluted with ethyl acetate, washed with saturated aqueous sodium hydrogen carbonate and water, dried over magnesium sulphate and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution (95:4.9:0.1) to yield the title compound (164 mg, 11%): MS (+ve ion electrospray) m/z 338 (MH^+).

Step 3. 4-[(3-*exo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yloxy] phenol - The product from Step 2 (164 mg, 0.49 mmol) was dissolved in dioxane (3 ml) and water (3 ml) and treated with 10% aqueous sodium hydroxide solution (0.5 ml). The mixture was stirred at room temperature for 3 hours and was then diluted with ethyl acetate and water. The organic

phase was separated and washed with water (x3), dried over magnesium sulphate and concentrated *in vacuo* to yield the title compound (73 mg, 64%): MS (+ve ion electrospray) m/z 234 (MH^+).

Reagent 2 - *N*-(2-Dimethylaminoethyl)-3-hydroxyisoxazole-5-carboxamide - Methyl 3-hydroxyisoxazole-5-carboxylate (2.0g, 0.013 mole) was added portionwise to a mixture of N,N-dimethylethylenediamine (1.52 ml, 0.013 mole) and methanol. The reaction mixture was stirred at room temperature for 16 hours, and then heated at 60°C for 3 hours. The solvent was removed *in vacuo*, and the residue purified by column chromatography, eluting with 5% methanol in dichloromethane, followed by and 20% (9:1 methanol/35% ammonia solution) in dichloromethane, to afford the title compound (2.43g, 96%) as a yellow gum: MS (+ve ion electrospray) m/z 200 (MH^+).

Example 301 Mutilin 14-(3-dimethylaminomethylphenoxy)acetate - 3-(Dimethylaminomethyl)phenol (R. Dillard *et al*, *J. Med. Chem.*, 1987, **30**, 911) (200mg, 1.3mmol) was dissolved in dry DMF (5ml), cooled to 0°C and treated portion-wise with sodium hydride (65mg of a 60% dispersion in oil, 1.3mmol). The mixture was stirred at 0°C for 30 minutes before the addition of pleuromutilin-22-mesylate (590mg, 1.3mmol). After stirring overnight at room temperature, the solvent removed and the residue partitioned between water and dichloromethane. The organic layer was dried (magnesium sulphate), concentrated and the residue purified by silica gel chromatography eluting with 1-5% (9:1methanol/35% ammonia solution)/dichloromethane to afford the title compound (250mg, 38%) as a cream foam: MS (+ve ion electrospray) m/z 512 (MH^+).

The following examples were prepared using the method described in Example 1.

Example Number	Name	Starting alcohol	Characterisation
302.	**Mutilin 14-[3-(piperidin-1-ylmethyl) phenoxy]acetate**	3-(1-piperidinyl-methyl)-phenol, *J. Org.Chem.*, 1959, **24**, 1952	MS, m/z 552 (MH^+)
303.	**Mutilin 14-(4-cyanophenoxy)acetate**	4-cyanophenol	IR (KBr), 3540, 2225, 1732 cm^{-1}
304.	**Mutilin 14-phenoxyacetate**	phenol	^1H NMR ($CDCl_3$) *inter alia*, 0.72 (3H, d, *J* 6.6Hz), 0.88 (3H, d, *J* 7.0Hz), 3.43 (1H, dd, *J* 10.8, 6.7Hz), 4.53 (2H, s), 5.20 (1H, d, *J* 17.3Hz), 5.35 (1H, d, *J* 10.9Hz), 5.87 (1H, d, *J* 8.5Hz), 6.49 (1H, dd, *J* 17.3, 10.9Hz), 6.85-7.03 (3H, m) and 7.20-7.31 (2H, m)
305.	**Mutilin 14-[4-(2-piperidin-1-yl-ethyl)phenoxy]acetate**	1-(4-hydroxyphenyl)ethyl-piperidine, *J. Am. Chem. Soc.*, 1951, 73, 4081	MS, m/z 566 (MH^+)
306.	**Mutilin 14-[4-(2-morpholin-4-yl-ethyl)phenoxy]acetate**	1-(4-hydroxyphenyl)ethyl-morpholine, *J. Am. Chem. Soc.*, 1951, 73, 4081	MS, m/z 568 (MH^+)
307.	**Mutilin 14-[4-(3-dimethylamino propyl) phenoxy]acetate**	4-(3-dimethylaminopropyl)phenol, *J. Am. Chem. Soc.*, 1964, **86**, 3075	MS, m/z 540 (MH+)

308.	**Mutilin 14-(4-dimethylaminomethyl phenoxy)acetate**	4-(dimethylaminomethyl)phenol, *J. Org. Chem.*, 1988, **53**, 4263	MS, m/z 512 (MH^+)
309.	**Mutilin 14-[4-(4-methylpiperazine-1-carbonyl)phenoxy] acetate**	1-(4-hydroxybenzoyl)-4-methyl-piperazine, *Eur. J. Med. Chem.*, 1996, **31**, 895	MS, m/z 581 (MH^+)
310.	**Mutilin 14-[4-(4-methylpiperazin-1-ylmethyl)phenoxy]acetate**	1-(4-hydroxybenzyl)-4-methyl-piperazine, *Farmaco,* 1992, **47**, 335	MS, m/z 567 (MH^+)
311.	**Mutilin 14-(4-formylphenoxy) acetate**	4-hydroxybenzaldehyde	^{1}H NMR ($CDCl_3$) *inter alia* 0.78 (3H, d, *J* 6.9Hz), 0.89 (3H, d, *J* 7.0Hz), 3.43 (1H, m), 4.63 (2H, s), 5.23 (1H, d, *J* 17.3Hz), 5.37 (1H, d, *J* 10.9Hz), 5.88 (1H, d, *J* 8.4Hz), 6.48 (1H, dd, *J* 17.3, 10.9Hz), 6.99 (2H, d, *J* 8.7Hz), 7.83 (2H, d, *J* 8.7Hz) and 9.90 (1H, s).
312.	**Mutilin 14-{4-[(3-*exo*)-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yloxy] phenoxy} acetate**	Reagent 1	MS, m/z 594 (MH^+).

313.	**Mutilin 14-(2-methyl-1,2,3,4-tetra hydroisoquinolin-7-yloxy)acetate**	2-methyl-7-hydroxy-1,2,3,4-tetrahydro-isoquinoline *Chem. Pharm. Bull.*, 1958, **6**, 497	MS, m/z 524 (MH^+).
314.	**Mutilin 14-(-2-methyl-1,2,3,4-tetra hydroisoquinolin-5-yloxy)acetate**	2-methyl-5-hydroxy-1,2,3,4-tetrahydroisoquinoline *Chem. Pharm. Bull.*, 1958, **6**, 497	MS, m/z 524 (MH^+).
315.	**Mutilin 14-(4-aminosulphonyl phenoxy)acetate**	4-hydroxy-benzenesulphonamide	MS, m/z 532 $(M-H)^-$.
316.	**Mutilin 14-(4-amino-2-dimethyl aminomethylphenoxy)acetate**	4-amino-2-dimethylaminomethyl phenol *J. Med. Chem.*, 1983, **26**, 1258	MS, m/z 527 (MH^+).
317.	**Mutilin 14-(2-dimethylaminomethyl pyridin-3-yloxy)-acetate**	2-(dimethylaminomethyl)-3-hydroxypyridine	MS, m/z 513 (MH^+).
318.	**Mutilin 14-[5-(2-dimethylamino ethylaminocarbonyl)isoxazol-3-yloxy]acetate**	Reagent 2	MS, m/z 560 (MH+).
319.	**Mutilin 14-[4-(2-dimethylamino ethylaminocarbonyl)phenoxy] acetate**	*N*-(2-dimethylaminoethyl) 4-hydroxy benzamide *Nucl. Med. Biol.*, 1997, **24**, 373	MS, m/z 569 (MH+).

320.	**Mutilin 14-(4-methoxycarbonyl phenoxy) acetate**	methyl 4-hydroxybenzoate	^{1}H NMR ($CDCl_3$) *inter alia* 7.98 (2H, d, J 8Hz), 6.89 (2H, d, J 8Hz), 6.47 (1H, dd, J 17, 12Hz), 5.86 (1H, d, J 8Hz), 5.47 (1H, dd, J 12, 2Hz), 5.20 (1H, dd, J 17, 2Hz), 4.59 (2H, s), 3.90 (3H, s), 3.34 (1H, m), 0.87 (3H, d, J 7Hz), 0.75 (3H, d, J 7Hz).
321.	**Mutilin 14-(2-ethoxycarbonyl-5-methylthiazol-4-yloxy) acetate**	ethyl 4-hydroxy-5-methylthiazol-2-carboxylate *J. Med. Chem.*, 1991, **34**, 2158	MS, m/z 548 (MH+).
322.	**Mutilin 14-(2-methoxycarbonyl thien-3-yloxy)acetate**	methyl 3-hydroxythiophene-2-carboxylate	^{1}H NMR ($CDCl_3$) *inter alia* 7.37 (1H, d, J 7Hz), 6.68 (1H, d, J 7Hz), 6.45 (1H, dd, J 17, 12Hz), 5.84 (1H, d, J 8Hz), 5.36 (1H, dd, J 12, 2Hz), 5.20 (1H, dd, J 17, 2Hz), 4.67 (2H, m), 3.85 (3H, s), 3.34 (1H, m), 0.86 (3H, d, J 7Hz), 0.73 (3H, d, J 7Hz).

Example 323 Mutilin 14-(4-carbamidoylphenoxy)acetate - Anhydrous hydrogen chloride gas was bubbled through a stirred solution of mutilin 14-(4-cyanophenoxy)acetate (Example 3; 294mg, 0.61mmol) and methanol (0.5ml) in dioxane (3ml). After 1 hour the solvent was evaporated and the residue triturated with diethyl ether giving a solid. The solid was dissolved in methanol (20ml) and ammonia gas was bubbled through the solution for 30 minutes. The mixture was then heated at 60°C for 16 hours under argon. After cooling to room temperature the solvent was evaporated and the residue purified by column chromatography on silica gel eluting with dichloromethane / methanol/ 35% ammonia solution (10:1:0.1 - 6:1:0.5) to afford the title compound (58mg, 20%) as a colourless solid: MS (+ve ion electrospray) m/z 497 (MH^+).

Example 324 Mutilin 14-[4-(2-dimethylaminoethyl)phenoxy]acetate

Step 1. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-bromoacetate - To a solution of (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin (500mg, 1.50 mmol) in dichloromethane (15 ml) at 0°C was added pyridine (0.143 ml, 1.76 mmol), followed by bromoacetyl bromide (0.133 ml, 1.52 mmol). The reaction mixture was allowed to warm to room temperature and stirred for a further 18 hours. The reaction was diluted with dichloromethane, washed sequentially with dilute hydrochloric acid and saturated aqueous sodium hydrogen carbonate solution, dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with 5% ethyl acetate in dichloromethane to yield the title compound (352 mg, 52%); ^1H NMR ($CDCl_3$) *inter alia* 0.81 (3H, d, *J* 6.6Hz), 0.99 (3H, d, *J* 7.0Hz), 3.21 (3H, s), 3.49 (1H, m), 3.82 (2H, s), 5.03 (1H, d, *J* 17.5Hz), 5.30 (1H, d, *J* 10.7Hz), 5.79 (1H, d, *J* 10.0Hz) and 6.61 (1H, dd, *J* 17.5, 10.7Hz).

Step 2. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[4-(2-dimethylaminoethyl)phenoxy]acetate - 4-(2-Dimethylaminoethyl)phenol (120mg, 0.73mmol) was dissolved in THF (10 ml) and treated with sodium hydride (29mg, 0.73mmol). The mixture was stirred at room temperature for 30 minutes and then cooled to 0°C. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-bromoacetate (300 mg, 0.66 mmol) was added and the mixture stirred at 0°C for 30 minutes and at room temperature for a further 18 hours. The mixture was diluted with ethyl acetate, washed with water, dried ($MgSO_4$) and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution

(95:4.9:0.1) to yield the title compound (338mg, 95%): MS(+ve ion electrospray) m/z 540 (MH^+).

Step 3. Mutilin 14-[4-(2-dimethylaminoethyl)phenoxy]acetate - The product of Step 2 (320mg, 0.59mmol) was dissolved in dioxane (2ml) and treated with conc. HCl (2ml). The mixture was stirred for 2 hours at room temperature and then poured into ethyl acetate and water. The aqueous layer was washed with ethyl acetate and then basified with aqueous potassium hydroxide solution. The product was extracted into chloroform (x2), dried ($MgSO_4$) and concentrated to yield a colourless foam (141 mg, 46%): MS(+ve ion electrospray) m/z 526 (MH^+).

Example 325 Mutilin 14-{4-[(3*S*)-(1-aza-bicyclo[2.2.2]oct-3-yl)minomethyl]phenoxy} acetate - The product from Example 11 (71 mg, 0.15 mmol) was dissolved in methanol (4 ml) and treated with (*S*)-(-)-3-aminoquinuclidine dihydrochloride (29 mg, 0.15 mmol) and triethylamine (0.021ml, 0.15 mmol). The mixture was stirred at room temperature for 6 hours and then treated with sodium triacetoxyborohydride (155 mg, 0.73 mmol). The reaction mixture was stirred for a further 14 hours and then diluted with dilute hydrochloric acid. This solution was washed with ethyl acetate (x3) and then the aqueous phase was basified with dilute aqueous sodium hydroxide solution. The product was extracted into chloroform (x2), dried over magnesium sulphate and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution (90:9.8:0.2) to yield the title compound (45 mg, 51%): MS (+ve ion electrospray) m/z 593 (MH^+).

Example 326 Mutilin 14-{4-[(3*R*)-(1-aza-bicyclo[2.2.2]oct-3-l)aminomethyl]phenoxy} acetate - The title compound (50 mg, 24%) was prepared from (*R*)-(+)-3-aminoquinuclidine dihydrochloride using the method described in Example 325: MS (+ve ion electrospray) m/z 593 (MH^+).

Example 327 Mutilin 14-(2-dimethylaminomethyl-4-methanesulphonamido phenoxy) acetate - The product from Example 16 (270 mg, 0.51 mmol) was dissolved in dichloromethane (10 ml) and cooled to $0^{o}C$. Methanesulphonyl chloride (0.043ml, 0.55 mmol) was added, followed by triethylamine (0.079 ml, 0.56 mmol). The reaction was allowed to warm to room temperature and stirred for a further 3 hours. The mixture was

diluted with dichloromethane, washed with water and brine, dried over magnesium sulphate and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution (90:9.8:0.2) to yield the title compound (226 mg, 73%): MS (+ve ion electrospray) m/z 605 (MH^+).

Example 328 Mutilin 14-benzyloxyacetate - Pleuromutilin (500mg, 1.3mmol) was dissolved in dry DMF (5ml), cooled to 0°C and treated portion-wise with sodium hydride (56mg of a 60% dispersion in oil, 1.4mmol). The mixture was stirred at room temperature for 30 minutes before the addition of benzyl bromide (0.17ml, 1.4mmol). After stirring overnight at room temperature, the reaction mixture was diluted with ethylacetate and washed with saturated aqueous sodium hydrogen carbonate, water and brine. The organic layer was dried over magnesium sulphate, concentrated *in vacuo* and the residue purified by silica gel chromatography eluting with 1:3 ethylacetate/hexane to afford the title compound (450mg, 76%) as a white solid: MS (EI) m/z 468 (M^+).

Example 329 Mutilin 14-(4-carboxyphenoxy)acetate - The product of Example 20 (500mg, 0.98 mmole) in 5:1 tetrahydrofuran/water was treated dropwise with 2M sodium hydroxide solution and the reaction mixture stirred at room temperature for 48 hours. The solvent was removed in vacuo, and the residue dissolved in water. The aqueous phase was acidified with 5M hydrochloric acid and extracted with dichloromethane. The organic extracts were dried ($MgSO_4$) and concentrated in vacuo. The product was purified by column chromatography, eluting with 1-2% methanol in dichloromethane, to afford the title compound (20mg, 5%) as a colourless gum: MS (+ve ion electrospray) m/z 497 (MH+).

Example 330 Mutilin 14-(2-carboxy-5-methylthiazol-4-yloxy)acetate - The title compound (120mg, 18%) was prepared from the product of Example 321 using the method of Example 329: MS (-ve ion electrospray) m/z 518 (MH-)..

Example 331 Mutilin 14-(2-carboxy-3-thienyloxy)acetate - The title compound (118mg, 10%) was prepared from the product of Example 322 using the method of Example 329: ^{1}H NMR (CDCl3) *inter alia* 7.52 (1H, d, J 7Hz), 6.73 (1H, d, J 7Hz), 6.44

(1H, dd, J 17, 12Hz), 5.89 (1H, d, J 8Hz), 5.36 (1H, dd, J 12, 2Hz), 5.20 (1H, dd, J 17, 2Hz), 4.70 (2H, s) 0.88 (3H, d, J 7Hz), 0.70 (3H, d, J 7Hz).

Example 332 Mutilin 14-[4-(2-dimethylaminoethylaminosulphonyl)phenoxy]acetate - The product from Example 315 (100 mg, 0.19 mmol) was dissolved in acetone (5 ml) and treated with 2-dimethylaminoethylchloride hydrochloride (27 mg, 0.19 mmol) and potassium carbonate (52 mg, 0.38 mmol). The reaction mixture was heated to reflux for 48 hours and then concentrated *in vacuo*. The residue was dissolved in ethyl acetate and washed with water and brine. The product was purified by chromatography on silica gel eluting with chloroform/methanol/35% ammonia solution (90:9.8:0.2) to yield the title compound (63 mg, 55%): MS (+ve ion electrospray) m/z 605 (MH^+).

Example 333 Mutilin 14-(2-methoxycarbonylphenoxy)acetate - Methyl salicylate (5 g, 33 mmol) was dissolved in dry DMF (100 ml) and potassium *tert*-butoxide (3.69 g, 33 mmol) added. After stirring for 20 minutes, pleuromutilin-22-mesylate was added and the mixture stirred for 16 hours. The mixture was then partitioned between diethylether and water. The diethylether layer was then dried (sodium sulfate), filtered and the solvent removed *in vacuo*. The residue was purified by silica gel chromatography eluting with ethyl acetate-hexane (6:4) to afford the title compound (3.15g, 56%) as a white foam: MS (-ve ion electrospray) *m/z* 571 $[M + O_2CCH_3]^-$

Example 334 Mutilin 14-(2-carbamoylphenoxy)acetate - The product from Example 333 (400 mg, 0.78 mmol) was dissolved in methanol (50 ml), cooled to –5 °C and then treated with gaseous ammonia for 5 minutes. The mixture was closed in a sealed tube and allowed to warm to room temperature, after which it was allowed to stand for 3 days. The solvent was removed *in vacuo* and the residue purified by silica gel chromatography eluting with ethyl acetate-hexane (1:1) to afford the title compound (172 mg, 44%) as a white foam: MS (-ve ion electrospray) *m/z* 556 $[M + O_2CCH_3]^-$

Example 335 Mutilin 14-(2-carboxyphenoxy) acetate - The product from Example 333 (1 g, 1.95 mmol) was dissolved in tetrahydrofuran-water (5:1) to which was added 1.98 M sodium hydroxide (0.98 ml). After 48 hours the reaction mixture was partitioned

between diethylether and 1 M aqueous sodium hydroxide. The aqueous layer was isolated and acidified with conc. hydrochloric acid until the product precipitated. The product was extracted into dichloromethane and the organic layer then washed with brine and dried (sodium sulfate). After filtration and removal of solvent, the residue was purified on silica gel eluting with ethyl acetate to afford the title compound (400 mg, 41%) as a white foam: MS (-ve ion electrospray) *m/z* 497 $(M - H)^-$.

Example 336 Mutilin 14-(isoquinolin-5-yl-oxy) acetate - 5-Hydroxyisoquinoline (153 mg, 1.05 mmol), DMF (10ml) and sodium hydride (42 mg of a 60% suspension in oil, 1.05 mmol) were stirred at 0 °C for 30 minutes. Pleuromutilin-22-mesylate (400 mg, 0.88 mmol) was then added and the stirred mixture allowed to come to room temperature over 2 hours. The solvent was removed *in vacuo* and the product partitioned between ethyl actetate and saturated aqueous sodium hyrogencarbonate. The organic layer was then washed with brine, dried (sodium sulfate), filtered and the solvent removed. Purification of the residue on silica gel eluting with ethyl acetate-hexane (2:1) afforded the title compound (100 mg, 22%) as a white foam: MS (+ve ion electrospray) *m/z* 506 (MH^+).

Example 337 Mutilin 14-[4-(2-*tert*-butoxycarbonylamino-2-methoxycarbonylethyl)-phenoxy] acetate - The title compound (1.44 g, 13%) was prepared from N-*tert*-butoxycarbonyl-D-tyrosine methyl ester using the method described in Example 333: ^1H NMR ($CDCl_3$) *inter alia* 0.74 (3 H, d, J 6.5 Hz), 1.42 (9 H, s), 3.45 (1 H, dd, J 10.7, 6.5 Hz), 3.70 (3 H, s) 5.84 (1 H, d, J 8.2 Hz), 6.79 (2 H, d, J 8.7 Hz), 7.02 (2 H, d, J 8.7 Hz).

Example 338 Mutilin 14-[4-(2-*tert*-butoxycarbonylamino-2-carboxyethyl)-phenoxy] acetate, ammonium salt - The product from Example 337 (1.68 g, 2.57 mmol) and lithium hydroxide hydrate (0.214 g, 7.71 mmol) were stirred in THF-H_2O (4:1) for 1 hour. The reaction mixture was partitioned between dichloromethane and 0.5 M hydrochloric acid. The organic layer was dried (sodium sulfate), filtered and the solvent removed *in vacuo*. Purification of the residue on silica gel dichloromethane/ methanol/ 35% ammonia solution (82:15:3) afforded the title compound (1.27 g, 75%) as a white solid: MS (+ve ion electrospray) *m/z* 659 $(M+NH_4)^+$.

Example 339 Mutilin 14-[4-(2-amino-2-carboxyethyl)phenoxy] acetate, ammonium salt - The product from Example 338 (1.42 g, 2.21 mmol) was dissolved in trifluoroacetic acid (36 ml). After 40 minutes the mixture was evaporated to dryness and the residue purified on silica gel eluting with dichloromethane/ methanol/ 35% ammonia solution (80:15:5) to afford the tile compound (0.84 g, 71%) as a white solid: MS (+ve ion electrospray) *m/z* 542 (MH^+).

Example 340 19,20-Dihydromutilin 14-[4-(2-carboxy-2-dimethylaminoethyl) phenoxy] acetate, ammonium salt - The product from Example 339 (0.78 g, 1.19 mmol), 37% aqueous formaldehyde (0.53 ml), DMF (1 ml), 5%-palladium/charcoal (50 mg) and ethanol (5ml) were stirred under a hydrogen atmosphere for 16 hours. The solution was then filtered and the solvent removed *in vacuo*. The residue was suspended in water which was adjusted to pH 7 using 2 M aqueous sodium hydroxide and the product then extracted with dichloromethane. The dichloromethane fraction was washed with brine, dried (sodium sulfate), filtered and the solvent removed *in vacuo*. Purification of the residue on silica gel eluting with dichloromethane/ methanol/ 35% ammonia solution (83:12:5) afforded the title compound (0.619 g, 91%) as a white solid: MS (+ve ion electrospray) *m/z* 572 (MH^+).

Example 341 Mutilin 14-[4-(2-amino-2-methoxycarbonylethyl)phenoxy] acetate hydrochlodide - The product from Example 337 (1.51 g, 2.30 mmol) was dissolved trifluoroacetic acid (25 ml). After 15 minutes the mixture was evaporated to dryness and the residue partitioned between diethylether and 0.5 M hydrochloric acid. The aqueous layer was evaporated to dryness to afford the title compound (0.715 g, 53%) as a white solid: MS (+ve ion electrospray) *m/z* 556 (MH^+).

Example 342 19,20-Dihydromutilin 14-[4-(2-dimethylamino-2-methoxycarbonyl-ethyl)phenoxy] acetate - The product from Example 341 (0.56 g, 0.957 mmol), 37% aqueous formaldehyde (0.43ml), 5% palladium/charcoal (50 mg) and methanol (15 ml) were stirred together under an atmosphere of hydrogen for 16 hours. The mixture was filtered and the solvent removed *in vacuo*. The residue was partitioned between dichloromethane and saturated aqueous sodium hydrogencarbonate. The organic fraction

was dried (sodium sulfate), filtered and evaporated to dryness. The residue was purified on silica gel eluting with diethylether-dichloromethane (1:4) to afford the title compound (0.111 g, 20%) as a white solid: MS (+ve ion electrospray) *m/z* 586 (MH^+).

Example 343 Mutilin 14-[5-(2-*tert*-butoxycarbonylaminoethyl)-2-methoxycarbonyl-phenoxy] acetate

Step 1. 5-(2-*tert*-butoxycarbonylaminoethyl)-2-methoxycarbonyl-phenol - 5-(2-aminoethyl)-2-methoxycarbonyl-phenol (S.F. Dyke *et al*; *Tetrahedron* 1973, **29**, 857) (1 g, 4.3 mmol), triethylamine (0.6ml, 4.3 mmol), di *tert*-butyl dicarbonate (0.942 g, 4.3 mmol) and DMF (40 ml) were stirred together at -20°C. The mixture was allowed to warm to room temp and stirred for 16 hours. The solvent was removed *in vacuo* and the residue purified on silica gel eluting with ethylacetate-hexane (1:6) to afford the title compound (720 mg, 47%) as a colourless oil: MS (-ve ion electrospray) *m/z* $(m-H)^-$.

Step 2. Mutilin 14-[5-(2-*tert*-butoxycarbonylaminoethyl)-2-methoxycarbonyl-phenoxy] acetate - The title compound (350 mg, 36%) was prepared from the product obtained from Example 343, Step 1 using the method described in Example 333. The product was purified on silica gel eluting with dichloromethane-acetonitrile (95:5) followed by dichloromethane–methanol (99:1): MS (-ve ion electrospray) *m/z* 714 $[M + O_2CCH_3]^-$.

Example 344 Mutilin 14-[5-(2-aminoethyl)-2-methoxycarbonylphenoxy] acetate - The product from Example 343 (0.57 g, 0.97 mmol) and 1:4 trifluoroacetic acid: dichloromethane, (15ml) were stirred for 1 hour after which the mixture was evaporated to dryness. The residue was partitioned between dichloromethane and saturated aqueous sodium hydrogencarbonate. The organic fraction was dried (sodium sulfate), filtered and evaporated to dryness. The product was purified on silica gel eluting with dichloromethane/ methanol/ 35% ammonia solution (90:9:1) to afford the title compound (326 mg, 66%) as a white foam: MS (+ve ion chemical ionisation) *m/z* 556 (MH^+).

Example 345 Mutilin 14-[5-(2-aminoethyl)-2-carboxy-phenoxy] acetate, ammonium salt - The product from Example 344 (60 mg, 0.0896 mmol) and sodium hydroxide (7.2 mg, 0.0896 mmol) were dissolved in THF: H_2O (4:1) and heated at reflux for 5 hours.

The solvent was removed *in vacuo* and the residue purified on silica gel eluting with dichloromethane/ methanol/ 35% ammonia solution (85:14.5:0.5) to afford the title compound (4 mg, 8%) as a white solid: MS (-ve ion mode) *m/z* 540 $(M-H)^-$.

Example 346 19,20-Dihydromutilin 14-[5-(2-dimethylaminoethyl)-2-methoxycarbonyl-phenoxy] acetate - The title compound (212 mg, 55%) was prepared from the product of Example 344 (350 mg, 0.65 mmol) using the method described in Example 342. The product was purified on silica gel eluting with dichloromethane-methanol (95:5): MS (+ve ion electrospray) *m/z* 586 (MH^+).

Example 347 19,20-Dihydromutilin 14-[5-(2-dimethylaminoethyl)-2-carboxy-phenoxy] acetate, ammonium salt - The title compound (55 mg, 35%) was prepared from the product of Example 346 using the method described in Example 345. The product was purified by silica gel chromatography eluting with dichloromethane/ methanol/ 35% ammonia solution (90:9:1): MS (+ve ion electrospray) *m/z* 572 (MH^+).

Example 348 19,20-dihydromutilin-14-[5-aminomethyl-2-methoxycarbonyl-phenoxy] acetate, hydrochloride

Step 1. Methyl 4-azidomethyl-2-hydroxybenzoate - Methyl 4-bromomethyl-2-hydroxybenzoate (1.96 g, 8.0 mmol) (*J. Am. Chem. Soc.* 1994, **116,** 2630), sodium azide (0.78 g, 12.0 mmol) and DMF (15 ml) were stirred together at 90 °C for 2.5 hours. The solvent was removed *in vacuo* and the residue partitioned between diethylether and water. The organic fraction was dried (sodium sulfate), filtered and evaporated to dryness to give the title compound (1.11 g, 67%) as a yellow oil: ^1H NMR ($CDCl_3$) 3.95 (3H, s), 4.34 (2H, s), 6.84 (1H, dd, J 8.1, 1.5 Hz), 6.94 (1H, s), 7.85 (1H, d), 10.82 (1H, s).

Step 2. Mutilin-14-[5-azidomethyl-2-methoxycarbonylphenoxy] acetate - The title compound (1.2 g) was prepared from the product obtained from Step 1 using the method described in Example 333. The product was purified on silica gel eluting with diethylether-dichloromethane (1:15): MS (+ve ion) *m/z* 590 (MNa^+).

Step 3. 19,20-Dihydromutilin-14-[5-aminomethyl-2-methoxycarbonylphenoxy] acetate, hydrochloride - The crude product from Step 2 (1.15 g, 2.03 mmol) was stirred with 5% palladium/charcoal (50 mg) in ethanol (60 ml) under a hydrogen atmosphere for 16 hours. The mixture was then filtered, evaporated to dryness and partitioned between

dilute aqueous hydrochloric acid and dichloromethane. The aqueous layer was evaporated to dryness to afford the title compound (150 mg) as a white foam: MS (+ve ion electrospray) *m/z* 581 (MH^+).

Example 349 19,20-Dihydromutilin-14-[5-dimethylaminomethyl-2-methoxycarbonyl-phenoxy] acetate - The title compound (0.18 g, 68%) was prepared using from the product of Example 348 using the method described in Example 342. The product was purified on silica gel eluting with methanol-dichloromethane (5:95): MS (+ve ion electrospray) *m/z* 572 (MH^+).

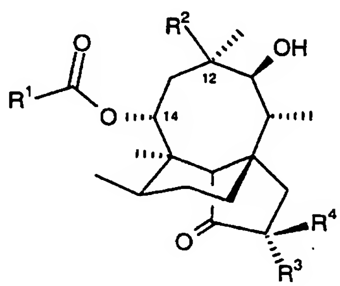
Biological Data

Compounds of the present invention were assessed for anti-bacterial activity in a conventional MIC assay against a range of pathogenic organisms.

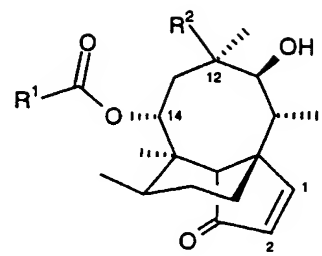
Compounds were found to have MICs in the range <0.06 to 32 μg/ml against *Staph Aureus* Oxford and <0.06 to 64 μg/ml against *Strep Pneumoniae* (r6).

CLAIMS

1. A compound of formula (IA) or (IB):



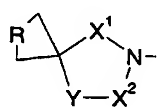
(IA)



(IB)

in which:

R^1 is $R^A(CH_2)_nO(CH_2)_m$, $R^A(CH_2)_p$, or



;

in which:

R is a spiro-fused mono- or bi-cyclic ring containing one or two basic nitrogen atoms; and X^1 and X^2, which may be the same or different, are each $-CH_2-$ or -C=O, provided that at least one of X^1 and X^2 is -C=O;

Y is -NH-, $-CH_2-$ or $-CH_2-CH_2-$;

R^A is an optionally substituted aryl group or heteroaryl group linked via a carbon atom;

m is 1, 2 or 3;

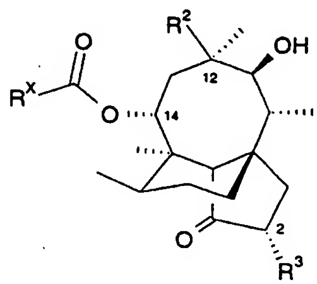
n is 0, 1 or 2; and

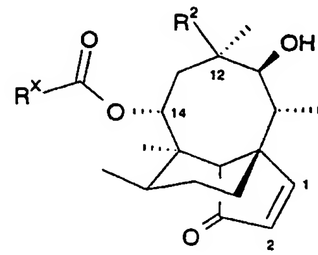
p is 1 to 4;

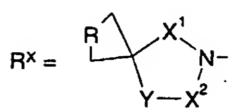
R^2 is vinyl or ethyl;

R^3 is H, OH or F, and R^4 is H, or R^3 is H and R^4 is F.

2. A compound according to claim 1 of formula (IA^1) or (IB^1):







(IA^1) (IB^1)

in which:

R^2 is vinyl or ethyl;

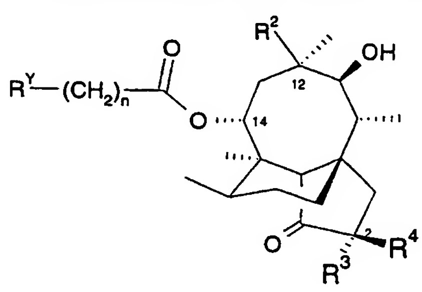
R^3 is H, OH or F;

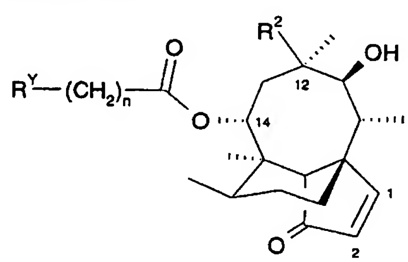
X^1 and X^2 which may be the same or different are each -CH_2- or -C=O, provided that at least one of X^1 and X^2 is -C=O;

Y is -NH-, -CH_2- or -CH_2-CH_2-; and

R is a spiro-fused monocyclic or bicyclic ring containing one or two basic nitrogen atoms.

3. A compound according to claim 1 of formula (IA^2) or (IB^2):





(IA^2) (IB^2)

in which:

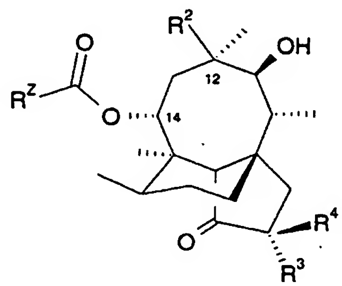
R^Y is an optionally substituted aryl group or heteroaryl group linked via a carbon atom;

R^2 is vinyl or;

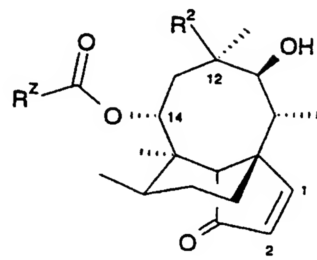
R^3 is H, OH or F, and R^4 is H, or R^3 is H and R^4 is F; and

n = 1 to 4.

4. A compound according to claim 1 of formula (IA^3) or (IB^3):



(IA^3)



(IB^3)

in which:

R^Z is a group $R^Y(CH_2)_nO(CH_2)_m$;

in which:

R^Y is an optionally substituted aryl or heteroaryl group linked via a carbon atom;

n is 0, 1 or 2; and

m is 1, 2 or 3;

R^2 is vinyl or ethyl,

R^3 is H, OH or F, and R^4 is H or R^3 is H and R^4 is F.

5. A compound according to claim 2 in which the ring R is piperidine or 1-azabicyclo[2.2.1]heptane, optionally N-substituted by (C_{1-6})alkyl or (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, or quinuclidine.

6. A compound according to claim 2 or claim 5 in which the ring containing X^1, X^2 and Y is pyrrolidinone, piperidinone or imidazolidone or the corresponding dione.

7. A compound according to claim 3 or claim 4 in which R^Y is optionally substituted phenyl, thienyl, pyridinyl, furyl, thiazolyl, isoxazolyl, benzimidazolyl, quinolinyl, 1,2,3,4-tetrahydro-isoquinolinyl or benzothienyl.

8. A compound according to claim 1 selected from

2,8-diaza-8-methyl-1-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester;

2,9-diaza-9-methyl-1-oxospiro[5.5]undecane-2-carboxylic acid mutilin 14-ester;
2,4,8-triaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-8-methyl-1,3-dioxaspiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-8-methyl-3-oxospiro[4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-1-oxospiro [4.5]decane-2-carboxylic acid mutilin 14-ester;
2,8-diaza-8-*tert*-butyloxycarbonylmethyl-1-oxospiro[4.5]decane-2carboxylic acid mutilin 14-ester;
(3*R*,4*S*)-spiro[(1-azabicyclo[2,2,1]heptane-3,3'-(2'-oxo-pyrrolidine)]-1'-carboxylic acid mutilin 14-ester;
(3*R*,4*S*)-spiro[(1-azabicyclo[2,2,1]-heptane)-3,3'-(2'-oxopyrrolidine)]-1'-carboxylic acid (2*S*)-2-hydroxy-mutilin 14-ester;
Mutilin 14-[4-(1-Methylpiperidin-4-ylmethyloxy)]phenylacetate;
Mutilin 14-(5-Dimethylaminomethyl-2-furyl)acetate;
Mutilin 14-[2-(piperidin-4-yl)thiazol-4-yl]acetate;
Mutilin 14-(4-dimethylaminomethylphenyl)acetate;
Mutilin 14-[3-(dimethylaminomethyl)phenoxy]acetate;
Mutilin 14-[4-(dimethylaminomethyl)phenoxy]acetate;
Mutilin 14-[4-(2-dimethylaminoethyl)phenoxy]acetate;
Mutilin 14-[4-(3-dimethylaminopropyl)phenoxy]acetate;
Mutilin 14-[4-(2-piperidin-1-yl-ethyl)phenoxy]acetate;
Mutilin 14-(2-methyl-1,2,3,4-tetrahydroisoquinolin-7-yloxy)acetate; and
Mutilin 14-[4-(2-dimethylaminoethylaminocarbonyl)phenoxy]acetate.

9. A method of preparing a compound according to any one of claims 1 to 8 which comprises introducing the C-14 side chain to mutilin or *epi*-mutilin by an appropriate coupling reaction, and if necessary converting the *epi*-mutilin to mutilin, and where necessary, before or after the coupling, modifying the mutilin nucleus to introduce 2-F; 2-OH; 19, 20-dihydro; or 1, 2-dehydro substituents.

10. A pharmaceutical composition comprising a compound according to any one of claims 1 to 8 or a compound obtainable by a process according to claim 9, or a pharmaceutically acceptable salt or derivative thereof, and a pharmaceutically acceptable carrier.

11. A method of treating microbial infections in animals, especially in humans and in domesticated mammals, which comprises administering a compound according to any one of claims 1 to 8 or a compound obtainable by a process according to claim 9, or a pharmaceutically acceptable salt or derivative thereof, or a composition according to the invention, to a patient in need thereof.

12. A method of treating or preventing recurrent otitis media or recurrent sinusitis in humans , which comprises nasally administering a compound according to any one of claims 1 to 8 or a compound obtainable by a process according to claim 9, or a pharmaceutically acceptable salt or derivative thereof, or a composition according to the invention, to a patient in need thereof.

13. A method of treatment of skin and soft tissue infections and in the treatment of acne in humans , which comprises topically administering a compound according to any one of claims 1 to 8 or a compound obtainable by a process according to claim 9, or a pharmaceutically acceptable salt or derivative thereof, or a composition according to the invention, to a patient in need thereof.